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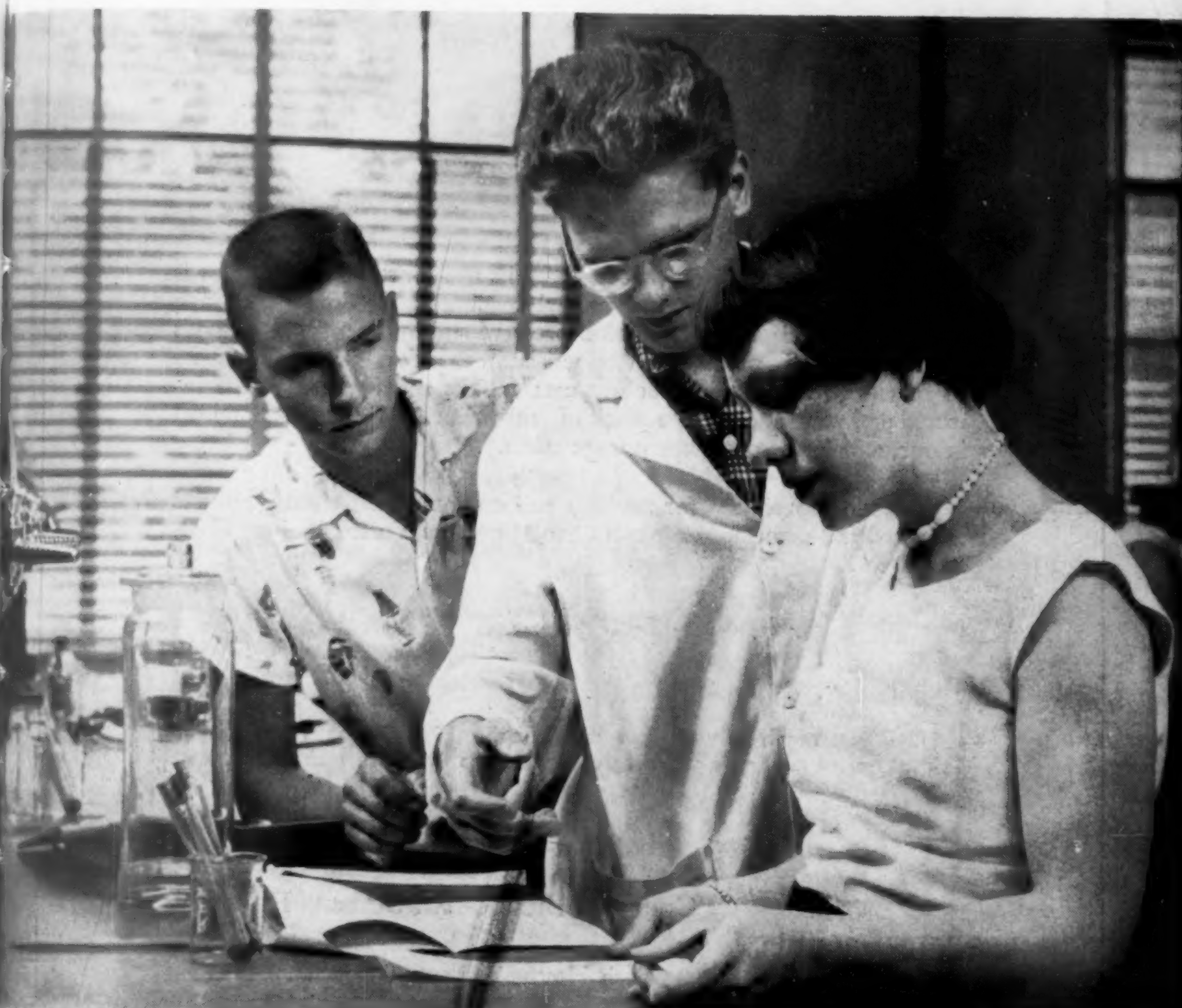

Suggestions for Laboratory Teaching

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THE AMERICAN BIOLOGY TEACHER

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Construction and Use of a Simple Ophthalmoscope[1]

THOMAS I. MARX, *Midwest Research Institute, Kansas City 10, Missouri*

Introduction

One of the simplest but most striking experiments within the scope of college and high school biology and physics courses is the examination of the human eye by use of the ophthalmoscope. There is no other way for the biology student to observe active blood vessels and nerves in the intact unanesthetized subject, and no subject is more convenient than the student's own lab partner. Physics students will find that the use of the ophthalmoscope will furnish many demonstrations in geometrical and physiological optics.

Professional ophthalmoscopes are obviously too expensive for school laboratories, and the Helmholtz-type instrument, while simple in principle, seldom yields successful results for the student. This report describes a simple yet effective ophthalmoscope for high school and nonmedical college students. It is hoped that the experience of observing blood vessels through the media of the eye will have an intrinsic educational value in addition to encouraging some students towards advanced study in physics and biology.

Instrument Design

The purpose of the ophthalmoscope is to illuminate the fundus or back of the eye in a way that will enable the observer to see the structures of the fundus while avoiding severe reflections from the cornea. The success of the instrument depends almost solely upon the geometry of the illumination system.

The optics of the ophthalmoscope system for two unaccommodated eyes is illustrated in Fig. I. The dotted lines show the extreme rays from the illumination cone that enter the subject's eye and illuminate a small circular area on the fundus. Some of the light diffused by the fundus reaches the observer's eye as indicated by the solid rays. It can be shown geometrically that light must be directed from a source, or an apparent source, between the subject and the observer near the latter's visual axis. Light falling into the subject's eye from the side of the observer's head is incident on the fundus at such a great angle that the illuminated area is invisible to the observer.

Proper fundus illumination can most easily be obtained by a semireflecting surface positioned coaxially with the observer's visual axis. This type of illumination, which was employed in Helmholtz's original ophthalmoscope, usually results in such severe light reflection from the subject's cornea that the observer's vision is obscured. However, if the illumination axis is positioned just below the observer's visual axis, cornea reflections in the center of the observer's view are eliminated entirely and the residual reflections, appearing at the edge of the field, can be minimized.

The illumination system of the ophthalmoscope consists of a General Electric No. 14 flashlight lamp, a 6 mm. diameter x 11.2 mm. focal length lens, and a small front surface mirror. If greater illumination is needed, as for example, when experimenting with polarizing filters to reduce cornea reflections, lamp No. 222 may be substituted. In general, however, the No. 14 lamp provides adequate illumina-

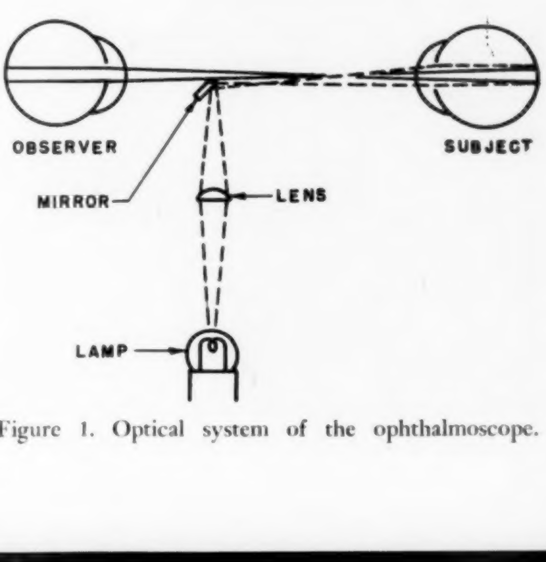

Figure 1. Optical system of the ophthalmoscope.

[1]Supported by grants from the National Science Foundation and the Basic Research Associates of the Midwest Research Institute, 425 Volker Boulevard, Kansas City 10, Missouri. Additional copies of this paper are available from the author.

tion but is not so bright as to disturb the subject; a few individuals may be unusually sensitive and should not be asked to act as subjects.

The lens is positioned 24 mm. from the lamp filament, the image of which is formed in space 21 mm. on the other side of the lens. The upper tip of the mirror is about 20 mm. from the lens and held at 45 degrees to the illumination axis. Some variation is possible in the dimensions of the illuminaton system. The important considerations are that the image of the filament is formed on or just beyond the mirror and that the illumination cone subtends an angle of 10 to 15 degrees.

Two student's ophthalmoscope models were made. Model I (Fig. 2), designed as a mass production prototype, is rugged in construction, and all parts are protected against accidental damage. The large, flat surface of the head is an aid to the inexperienced observer who gains a sense of direction as he handles the instrument. The observer's aperture is 3 mm. (⅛ in. nominal). A "front aperture" is effectively formed by the upper edge of the illumination mirror and the top edge of the front hole; the observer automatically centers and aligns these apertures to his own visual axis.

Figure 2. Model I Student's Ophthalmoscope for commercial production.

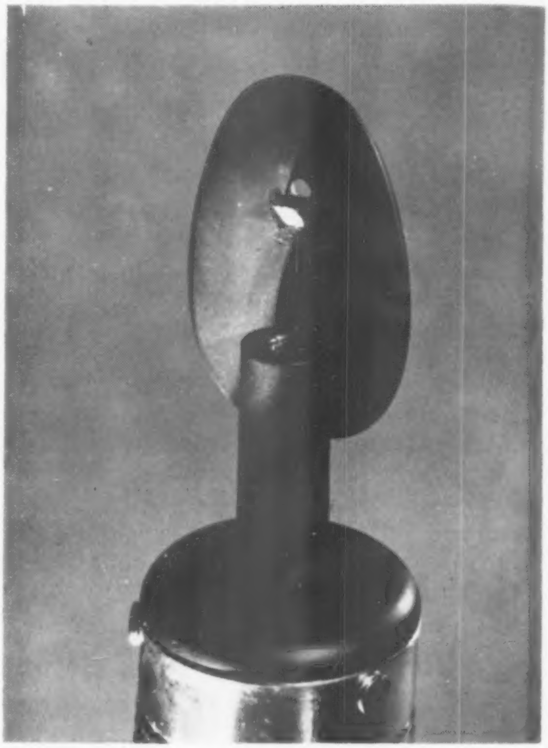

Figure 3. Model II Head for teacher-student construction.

Model II (Fig. 3), designed for manual construction by teachers and students, is optically identical to Model I. The head is simple to fabricate by hand, but the lens and mirror are vulnerable to damage. Because of the single aperture and the smaller head surface, the observer's sense of direction with the Model II is not as good as with Model I. In general, however, the two models are almost equal in performance.

Construction Details of Model II

Dimensions for constructing the Model II head are shown in Fig. 4. The aperture plate is 1/16 or ⅛ lucite sheet, the neck is ½ in. lucite rod 1⅝ in. long, and the cap is made from lucite. The handle is made by cutting down a commercial flashlight, care being taken not to break off the base-contact strip. The lamp socket, a miniature screw base socket obtained from a radio parts supply house, is held to the bottom of the cap by a screw that passes through the base contact lug. When assembled

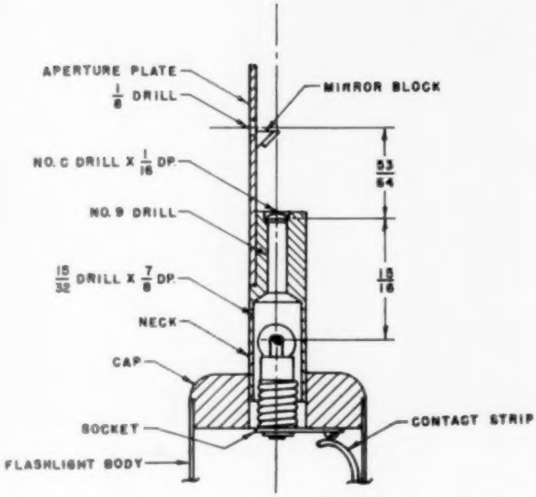

Figure 4. Construction drawing of Model II Head.

onto the handle, the base-contact strip of the flashlight body contacts this screw, and the positive terminal of the top flashlight cell contacts the center of the socket. The external surfaces of the head are sprayed with flat black paint, and the internal surfaces of the neck are coated several times with Kodak Brushing Lacquer or equivalent.

The lens, from Edmund Scientific Company, Barrington, New Jersey, is mounted in the top of the neck by a few tiny drops of Duco cement around the edge. The aperture plate is cemented with Duco to a flat filed into the neck.

Placement of the mirror is the most critical part of the construction. A 4 x 6 mm. piece is cut from a 1 mm. thick front surface mirror, Edmund Scientific Company, and one short edge is beveled at 45 degrees on a coarse stone flooded with water. The mirror is mounted with Duco to a small lucite 45-degree angle block which is cemented to the aperture plate with Duco. Final positioning of the mirror is completed before the cement sets. The lamp is turned on and the mirror adjusted until its upper tip just intersects the outer edge of the illumination cone. The mirror block is then moved so that the mirror's upper tip "cuts-off" the lower ½ to 1 mm. of the aperture. By thus utilizing the uppermost tip of the mirror and bringing the illumination axis into near coincidence with the visual axis, the largest possible fundus area can be observed. The edges of the mirror and holder are coated with Kodak Brushing Lacquer.

Model Eye

A few minutes' work with a Model Eye is of great aid to the student who has difficulty seeing the human ocular fundus. The Model Eye (Fig. 5) is made by cementing a lens to a 1 in. diameter hollow plastic toy ball. Although the human eye is most accurately represented by a 15 mm. focal length lens in air, the Model Eye was designed with a 24 mm. lens as the larger unit is easier to handle.

The lens is mounted with Duco cement in a 5/16 in. drilled hole in the ball, and the opposite end of the ball is cut down so that the plane of the cut section falls just inside the focal point of the lens. The Model Eye is thus slightly hypermetropic, farsighted; such a condition is helpful to inexperienced observers who often tend to accommodate their eyes rather than "relax" them.

The fundus is represented by a drawing cemented to the back of the ball. A few lines are used to represent blood vessels and a 2½ mm. diameter circle represents the optic disc. If a different focal length lens is used for the eye, the diameter of the optic disc should be corrected. The human eye has a focal length of about 15 mm., and the diameter of the optic disc is 1.5 mm.

Because of the large aperture the fundus of the Model Eye is easy to observe. Once the student has become familiar with the ophthalmoscope and observation of the Model Eye, his observation of the human eye is greatly simplified.

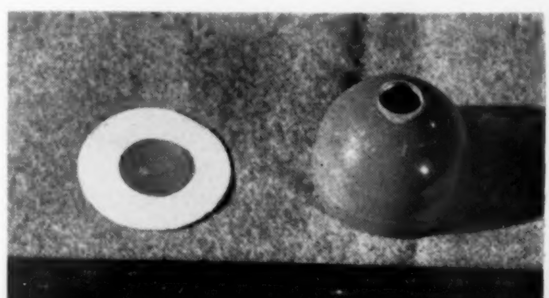

Figure 5. Model Eye, sub-assembly view.

Use of the Ophthalmoscope

The ophthalmoscope is most easily used in a darkened room. An ordinary classroom with shades drawn and room lights turned off is usually dark enough. If the room cannot be sufficiently darkened, the first position, below, should be eliminated.

First position: The subject is seated, preferably facing a dark wall. The observer sits or stands slightly to one side, at a distance of about ¼ to ½ m. The subject is told to look at a fixation point directly in front of him and at a distance of at least 7 m. If the observer prefers to use his right eye, he should examine the subject's right eye. The observer stands slightly to the right of the subject, and the latter's line of sight from his right eye to the fixation point should pass just outside the observer's right ear. The observer should use his left eye to examine the subject's left eye, and he should stand to the subject's left.

The observer then holds the ophthalmoscope to his eye. He is instructed to keep both eyes open and to relax his accommodation as if he were gazing at a distant object. While looking through the aperture, he then maneuvers the ophthalmoscope until light falls into the subject's eye at which time he will see a red spot in the subject's pupil. This red spot, called the red reflex, indicates that light is properly entering the subject's eye.

Second position: Once the red reflex has been found, the observer approaches the subject maintaining the red reflex at all times. He must be cautioned against accommodating, or trying to "look at" the subject's eye, and the subject likewise must be instructed to remain gazing in the direction of the fixation point even though the observer's head may interrupt his vision. As the eye-to-eye distance shortens, the observer will see a few dark lines in the pupil. These are fundus blood vessels, and as the observer moves closer more vessels will become visible. To obtain the greatest field, the observer is instructed to move as close to the subject's eye as possible without touching the latter's eyelashes (Fig. 6). Unless the subject's pupil is contracted, 2 mm. or more of the fundus should be visible.

The observer may direct his vision and the ophthalmoscope to view various parts of the fundus (Fig. 7). The optic disc, contain-

Figure 6. Second position: examination of the ocular fundus.

ing the optic nerve and a number of intersecting blood vessels, is most easily recognized. It is slightly nasal to the eye's center line and is therefore viewed at an angle as if the observer were looking towards the rear-center of the subject's head. The arteries may be identified as being slightly smaller in diameter than the veins; the reasons for this difference involve several aspects of the physics of the circulatory system which the student should be encouraged to study. In any case, the student may appreciate the fact that he is observing essentially "exposed" vessels without the use of surgery.

After the observer has gained competence in viewing the fundus at various angles, he may wish to find the *fovea centralis*, or center of vision. This should be done at the end of the examination, since the fovea is most sensitive to light, and slight discomfort and constriction of the pupil may result. The eye is an unique optical device, since the optical axis is not utilized as the visual axis. The fovea lies more than a millimeter to the temporal side of the second focal point of the eye. When the observer looks into the subject's eye at an angle as if he were looking in the direction of the subject's ear, he will see a tiny white dot in the center of an area noticeably void of blood vessels. This area is called the macular area, and the white dot is the fovea centralis, consisting entirely of retina cones.[2] Except for the dark-adapted eye,

[2]The fovea is most easily recognized in young subjects; the "white dot" fades with age and may not appear in normally sighted adults.

visual acuity is greatest at the fovea, and the eye automatically positions the image of whatever object it is "lookir.g at" on the fovea. For further inquiry certain students may be encouraged to pursue studies of visual stimulation and perception and possibly binocular vision.

Use of an Ophthalmoscope without Correction Lenses

The elimination of the wheel of twenty or more correction lenses found in professional instruments has greatly simplified the student's ophthalmoscope. The physician uses these lenses to compensate for the ametropia of the subject, and possibly himself. However, when no lenses are used, and the subject is more hypermetropic, farsighted, than the observer, the latter can usually use his power of positive accommodation to compensate for the difference. If the observer is myopic, nearsighted, or strongly hypermetropic, he can wear his spectacles for correction. If the subject is very myopic, the use of his spectacles will usually facilitate observation, although satisfactory results may be impossible. It will be noted here that the observer cannot correct for the subject's myopia, since negative accommodation is physiologically impossible.

Consideration of these conditions leads to the conclusion that in groups of students all persons may use the ophthalmoscope for observation. However, in severe cases the most myopic individual in the group may not be able to serve as a subject. This is not a serious limitation since it is the observer who is receiving the educational instruction.

Additional Experiment to Demonstrate Accommodation

The student may use a 5 to 15 cm. focal length lens to demonstrate the principles of accommodation. While relaxing his accommodation, the student positions the lens to clearly focus an object at reading distance. The lens is then moved closer to the object, and the observer will note that he can bring the object back into focus by employing positive accommodation. The eye is relaxed, and the lens is again adjusted to focus the object.

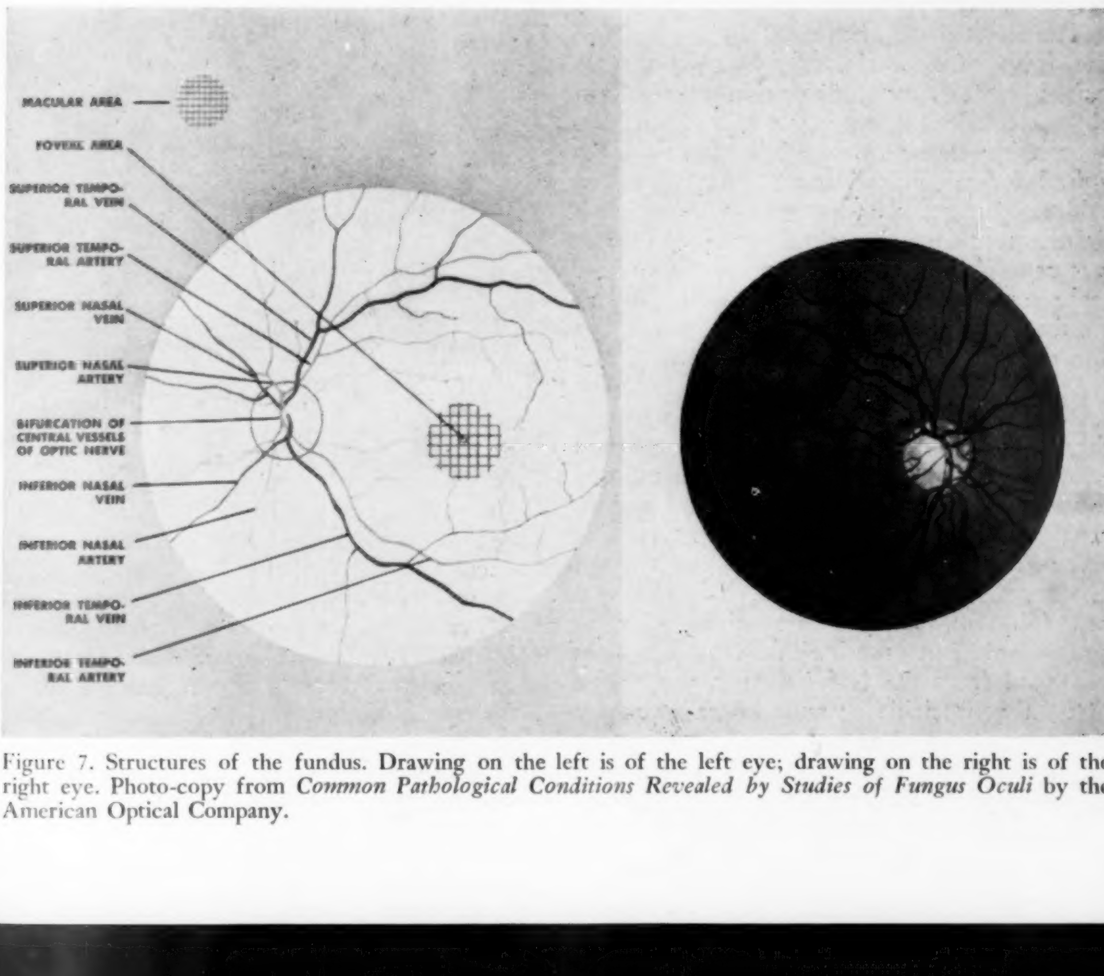

Figure 7. Structures of the fundus. Drawing on the left is of the left eye; drawing on the right is of the right eye. Photo-copy from *Common Pathological Conditions Revealed by Studies of Fungus Oculi* by the American Optical Company.

The lens is now moved more distant from the object, and the student will observe that the object cannot be brought into focus since negative accommodation is physiologically impossible. The eye-to-object distance remains fixed during the entire experiment.

Virus Reproduction

Experiments designed to test a new concept of virus reproduction were reported by group of investigators headed by Dr. Barry Commoner, professor of plant physiology at Washington University, St. Louis. The experiments were done with tobacco mosaic virus, a submicroscopic rod-shaped particle, which is reproduced in leaves of infected tobacco plants.

The theory that the investigators are testing, developed on theoretical grounds last year by Dr. Commoner, proposes that the virus is reproduced by a kind of linear growth at one end of the rod, with both of the virus' chemical constituents—protein and ribonucleic acid —participating in the reproductive process. Earlier theories have stressed the idea that only the ribonucleic acid (RNA) reproduces itself, the protein being later synthesized by a process controlled by the ribonucleic acid.

The RNA of tobacco mosaic virus exists in the form of a single, and Dr. Commoner pointed out that such a single thin strand of nucleic acid cannot by itself maintain the definite geometrical arrangement that appears to be required for self-reproduction. The RNA can achieve the necessary structure only when tightly bound to the virus protein. Since it was recently discovered that some forms of reproductive desoxyribonucleic acid (DNA) consist of a single strand the new theory may also suggest a means for reproduction of this type of DNA.

In one set of experiments to test the new theory, tobacco leaves that had been infected with the virus were illuminated and exposed to radioactive carbon dioxide for brief periods ranging from 3 to 45 minutes. The radioactive carbon dioxide was rapidly assimilated through photosynthesis and converted into the raw materials required for the synthesis of virus nucleic acid and protein. The virus was then extracted from the leaf tissue; it was found that an appreciable amount of radioactivity had entered the virus rod.

These radioactive rods were then shortened in a step-wise fashion by the use of a technique for the gradual removal of small amounts of protein and RNA from one end of the rod, thus reducing its length by a measurable amount. When successively longer bits were removed from the virus rods and analyzed they were found to show considerably more radioactivity than the whole virus. Since the radioactivity of material available in the leaf for virus synthesis increases during the course of the experiment, any difference in radioactive carbon content among different bits are synthesized from raw material at different times. The analyses showed that the radioactivity—and therefore the newly synthesized material—is localized near one end of the rod and that as the experimental time increased the radioactive material moved into the deeper portions of the rod. From the rate of this movement, it was concluded that in the infected leaf, about 10-30 minutes is required to form a virus rod.

This result means that in the infected leaf the virus is synthesized by the progressive addition of new materials to the end of the growing rod. Separate analysis of the radioactivity of the virus protein and RNA showed that this conclusion applied equally well to both virus constituents. These results support the new theory in showing that virus formation occurs by a kind of linear growth in which both virus protein and RNA are produced simultaneously. The earlier theory would require that virus RNA be synthesized ahead of the protein and that no linear distinction in radioactivity should be observed.

Measures and Weights

One of the really fine publications that will be of interest to high school science teachers, but particularly to the junior and elementary school teachers, is the *Kansas School Naturalist*, published by the Kansas State Teachers College of Emporia. The Editor is former *ABT* Editor, Professor John Breukelman, Department of Biology, at Kansas State. The latest issue, April, 1960, is entitled "Measures and Weights." This is a fine publication to have sent to you regularly.

Studying the Effect of Various Crude Drug Media on the Cultivation of Fungi

ARNOLD I. MILLER, *Thomas Jefferson High School, Brooklyn, New York*

One of the problems facing educators today is finding suitable projects for gifted students. Many problems are either too difficult or not challenging enough. Those that are acceptable involve expensive materials which are often difficult to obtain.

This past summer I participated in a research participation program for high school teachers under a National Science Foundation grant at Saint John's University. My research participation involved the field of microbiology, particularly mycology, and was directed by Dr. Michael A. Pisano of the Biology Department.

The project lends itself beautifully to individual and/or group work in the high school laboratory. It is neither too easy nor too difficult for able pupils. The materials are easily obtainable and are comparatively inexpensive. Results can be obtained within twenty-four hours after inoculation, with the entire duration of the project lasting at least two weeks. High school students experience greater pleasure and exhibit a higher level of interest when they can actually see results from their work in a short period of time. This project is especially adapted to this factor.

By adding various crude drug extracts to basic fungal media many interesting and unusual observations can be made. This experience can also be performed with bacteria as the organisms of choice. The simplified procedure is as follows:

The amounts given below are for 250 cc. of the media. The figures can be adjusted to suit individual needs.

1. 25 grams of a crude drug, various common spices and medicinal products, are macerated thoroughly using a mortar and pestle; an electric food blender, if available, is ideal.
2. 250 ml. of tap water is then added to the crude drug and thoroughly mixed.
3. The entire mixture is brought to boiling and allowed to simmer for twenty minutes.
4. Cool the mixture to room temperature.
5. If a centrifuge is available, centrifuge the mixture at 2000 r.p.m. for twenty minutes. If a centrifuge is not on hand, allow the coarse particles to settle and pour the supernatent liquid into a 250 ml. graduate.
6. Restore the volume of the supernatent liquid to 250 ml. using ordinary tap water.
7. At this point, the crude drug extract can be placed in a bottle and stored in a freezer until needed. Keep the cap on the bottle-top loose, or else the expanding water in it might crack the bottle. Remember to bring the extract to room temperature before performing any work on it.
8. Filter the extract to remove any coarse particles.
9. A pH reading is then taken using pH paper. If the pH is 4.5 or below, adjust with sodium hydroxide, or the agar will not gel.
10. 1.5% agar (3.75 grams) and 1.0% dextrose (2.5 grams) are added to the extract.
11. The agar and dextrose are brought into solution by boiling.
12. Dispense the finished extract into test tubes and sterilize at 121° C. for fifteen minutes. Slant tubes which after cooling can be inoculated.

The fungi are inoculated onto the slant using the regular mycological procedure. The results are compared with the growth of fungi on Saboraud-Dextrose agar, the control, the most widely-used fungal culture medium. Following inoculation the cultures should be kept in an incubator to insure a constant temperature.

The choice of types of fungi to be utilized is left to one's own discretion. However, during the summer the fungi employed in our experiment included two representatives from each of the five groups, as follows:

Yeasts
Nematospora coryli
Saccharomyces cerevisiae
Basidiomycetes
Poria johnsoniana
Coprinus radians

Phycomycetes
Rhizopus arrhizus
Cunninghamella echinulata
Deuteromycetes
Fusarium solani
Cephalosporium ciferri
Ascomycetes
Thielavia terricola
Fimetaria humana

Charles Pfizer and Company supplied all of the above cultures, except for *Cephalosporium ciferri*, which was obtained from the university stock. The cultures were kept on petri dishes of Saboraud-Dextrose agar from which the transfers were made and stored in an incubator.

Readings were made one, three, seven and fourteen days following the original transfers as to surface growth in the test tubes as follows:

#X = No growth.
#0 = Trace of growth.
#1 = Growth 1-25% of the slant.
#2 = Growth 26-50% of the slant.
#3 = Growth 51-75% of the slant.
#4 = Growth 76-100% of the slant.

Characteristics such as type of colony, pigments produced, if any, were also recorded. Pay particular attention to any inhibitory effect that the medium may exhibit.

Some crude drugs that are easily obtainable, inexpensive and show interesting results are as follows:

Clove	Black Pepper
Vanilla Bean	White Mustard Seed
Allspice	Poppy Seed
Rosin	Spearmint Leaves
Caraway Seed	Celery Seed
Peppermint Leaves	Sage
Anise Seed	Bay Leaves

Not only does this project afford the student with a valuable experience in biology, but also shows him what a project in research is actually like. This particular area of biology is neglected and passed over by many scientists. Too many high school students are under false impressions that research is characterized by great and spontaneous discoveries, one after another. This problem-solving experience presents present-day research as it really exists—with its routines and relatively small discoveries, adding small colors, little by little, to fill the entire canvas of the painting as a whole.

Acknowledgments

I wish to thank the following people who have made this summer's experience so valuable for me:

Dr. Michael A. Pisano, head of the above project.

Dr. Paul T. Medici, head of the N.S.F. program at Saint John's University.

Dr. Daniel M. Lilly, Chairman of the Biology Department, Saint John's University.

Mr. Jerome Berger, Teacher of Biology, Plainview High School, Plainview, New York, who was my co-worker on this project.

Mr. Milton Lesser, Chairman of Science Department, Thomas Jefferson High School, Brooklyn, New York, who called this wonderful program to my attention.

European Travel Study Program In Comparative Education

Wayne State University's College of Education and Graduate Division again approves credit arrangements in connection with the 14th Annual European Travel Study Program in Comparative Education. Personally directed by Dr. Wm. Reitz, Professor of Education, the group will leave Detroit on June 24 and return on September 1, 1961.

Visiting 13 countries during the 10 weeks' journey, this program is designed to provide teachers, students, and other professional people with opportunities to survey selected highlights of the life and culture of Western Europe.

Special highlights of the 1961 program include an Adriatic and Aegean cruise visiting ports of Greece and Turkey: Corfu, Athens, Mykonos, Delos, Istanbul, Bosphorus, Troy, Rhodes, Crete, Delphi, Nauplia, and Brindisi.

Further information may be obtained from Dr. Wm. Reitz, College of Education, Wayne State University, Detroit 2, Michigan.

Conservation

A new publication, "Research in Wisconsin, 1958-1959," is available from the Wisconsin Conservation Department, Madison 1, Wisconsin. It is a technical digest of research results and fish management, forestry, and game management. It has been prepared by John E. Beale, Chief State Forester.

A Simplified Method of Drosophila Culture for the Classroom

JACK BENNETT
Northern Illinois University, DeKalb, Illinois

Many high school and college instructors have attempted to use the common fruit fly, *Drosophila melanogaster* Meigen, in the elementary biology classroom, usually to illustrate some simple Mendelian ratios. Many have also given up after one such attempt and advised others to avoid the beasts. The purpose of this article is to describe and illustrate some simplifications of technique and equipment which have, in the author's experience, eliminated many objections to the use of Drosophila in the general biology class.

One of the greatest barriers has been the preparation of the food media. The difficulty has resulted from the very refinement of the techniques used in advanced college and research laboratories. The teacher who has had a laboratory course in genetics has been taught that these animals must be fed on a special formula. Those who have prepared Drosophila media know that making a baby's formula is simple by comparison. The synthetic food media, while they do have a legitimate place, are out of place in the general biology laboratory.

Any teacher, or student, can make first-rate fly food in a few minutes with only three ingredients, all of which are available at any grocery. One needs: 1. A banana, preferably barely ripe and with no soft spots that could contain fly larvae; 2. A five-cent envelope of dried, granulated yeast; 3. A box of facial tissue.

Select the glassware to be used (more on this point later), making sure that it is clean. Peel the banana, discard the skin, and mash to a soupy pulp. Spoon the banana mash into the glassware, to a depth of ½″ to ¾″. Make a wad of tissue large enough to occupy about one-fourth of the bottom of the container, and push it into the banana mash at one side. The paper soaks up excess moisture and provides a resting place and site for pupation. Then shake a few grains of yeast, not more than 3 or 4 per square inch of food area, on to the mash. A cotton plug in the mouth of the container completes the process. The result is a few containers of the most nutritious fly food available (Figure 1).

Care should be taken in the selection of the bananas, for one fly larva in a soft spot could contaminate a carefully kept strain and render it useless. For most laboratory strains of flies the contamination would not be too catastrophic but would require careful attention to the strain for a couple of generations.

Why isn't this method used in research laboratories? It is and has been used. It is the method generally used during the first few years of laboratory research with Drosophila. It is being used in research in genetics and

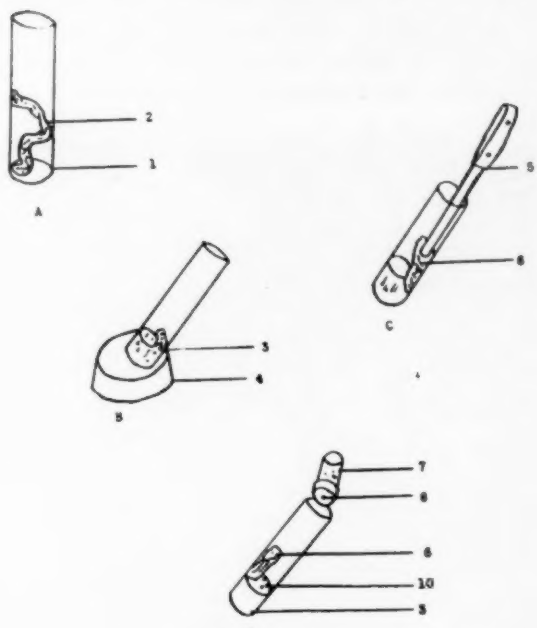

Figure 1. Stages in preparation of vial of banana food. A. Vial with extruded banana. B. Vial bounced to jar banana into uniform mass at bottom. C. Spatula pushing wad of tissue into food mass. D. Shaking granulated yeast onto food. 1. 25 x 95 mm. glass vial. 2. Ribbon of extruded banana. 3. Banana food compacted. 4. No. 14 rubber stopper. 5. Spatula. 6. Strip of cleansing tissue. 7. Shaker with yeast. 8. Single opening to limit yeast flow. 9. Grains of yeast on food.

evolution in the Drosophila Laboratory of the Northern Illinois University Department of Biological Sciences. The method is a time saver and produces as large and healthy flies as the author has seen produced on any food medium.

Why isn't it used more commonly? There are several reasons. For many, but by no means all, research projects the banana food is too variable. Thus, the less nutritive but less variable synthetic media may be required. It is at its greatest advantage where only small amounts of food are needed at one time, as in the classroom or small laboratory. When several quarts, or gallons, of food are used each day it is generally cheaper and faster to prepare synthetic media. When only ounces of food are needed, and then only once a week, it is the method of choice. One banana will make up about 10 or 12 small vials of fly food, usually a convenient amount. Excess can be stored in the freezer of the refrigerator for use later. Finally, few people have ever heard of using bananas, directly, for fly food.

With banana mash food it is easy to keep a supply of flies in the classroom all year long. No elaborate equipment is required and the expenses run to 15 or 20 cents a month to maintain up to a dozen different strains of flies. Most any budget will support this outlay.

The author has found it convenient, though not necessary, to use some small items of equipment, costing less than a dollar, in the regular use of banana mash food. A small aluminum cake decorator, Figure 2, has been used to mash the banana and inject it into the food vials. One of the spouts was enlarged to about ¼″ opening by forcing a pencil through it. The banana is mashed as it is forced through this opening and can be directed into a vial without smearing the mouth of the vial. After a year's heavy use, including one semester in a classroom, the original cake decorator wore out. It was replaced by a standard caulking gun, Figure 3, which also works very well and takes a larger load of banana.

A small salt shaker, modified by closing all but one or two holes with glue, is very useful for holding the granulated yeast and shaking it into the vials. These items, which can be found in most kitchens or dimestores, are helpful and do not require extensive space.

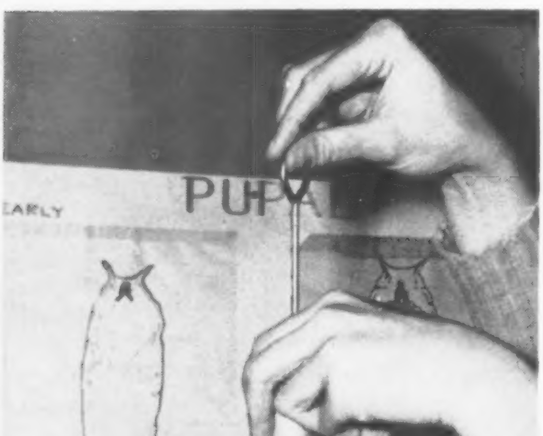

Figure 2. Cake decorator in use, extruding ribbon of banana mash into vial.

Figure 3. Caulking gun in use, extruding ribbon of banana mash into vial.

Traditionally, the glassware for raising Drosophila has been the ½ pint milk bottle. For most purposes it is really much too large. A common laboratory test tube is a more useful size for classroom purposes, but it has the disconcerting habit of rolling off a table and breaking.

The small glass jars used for many kinds of baby food have a large mouth, relatively straight sides, and a small total volume. They are usually available in quantity from the parents of small children. They can be satisfactorily used with banana mash food.

Almost any kind of bottle or jar can be used. The author has even used one-gallon milk bottles with varying degrees of success. If possible, however, the author advises the purchase of 1″ x 4″ (25 x 95 mm.), straight sided, glass shell vials (8 dram size) such as Kimble #60930. These are sold by most supply houses at $4.95 per gross. One such vial will raise about all the flies a beginning student can count accurately in an hour. It is a great advantage to use containers of the same size for all purposes; then flies can be easily transferred from one to another without loss.

Those who have worked with Drosophila may ask, "What happens when you invert the vials and shake the flies out? Doesn't the soft banana mash come with them?" The answer is no, because it isn't necessary to shake the vial violently or jar it on a table top. Two good alternate methods are available.

If only a few flies are to be handled, one may gently jar them down onto the food, remove the cotton plug, and invert a clean vial over the top. With good overhead light, the flies will climb upward into the clean vial, which may then be raised and plugged. If a large number of flies are to be handled, the flies can be etherized. An ideal device for this purpose was described in the ABT by Arnold in 1957. It consists of a squeeze-bottle dispenser with a small pointed tip. The bottle is loosely stuffed with cotton partially wet with ether. A polyethylene catsup dispenser with a long pointed tip is fine. The hypodermic needle suggested by Arnold is unnecessary for this purpose. In use one inverts the vial, inserts the pointed tip beside the cotton plug, and squeezes ether vapor up into the vial (Figure 4). The etherizer must be removed before letting it refill with air or

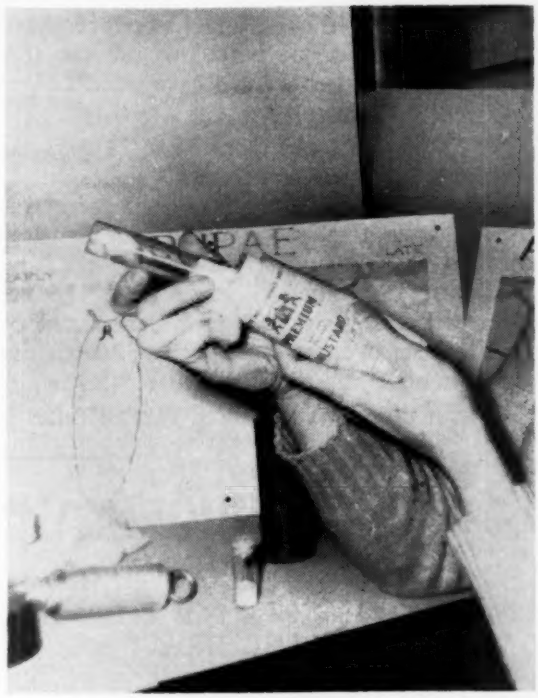

Figure 4. Etherizer in use. Ether vapor is expelled past cotton plug into vial to anesthetize the flies, which fall on cotton.

the flies may be drawn into it. After a few seconds the flies will become unconscious and fall onto the cotton plug. When all have succumbed remove the cotton plug and allow the flies to fall onto a clean sheet of paper. The author ordinarily uses a 3″ x 5″ card to collect the flies. It can easily be curved into a trough for shaking them into another vial after examination. The polyethylene etherizer described above is the best classroom etherizer the author has seen and is usually available for 25 cents or less.

These methods have made it possible for many students to study Drosophila in the classroom and at home. They have saved much time and anguish on the part of the biology teacher and thereby made possible better classroom instruction in heredity, evolution, and the study of an insect life cycle.

The only variation from breeding procedures used with the synthetic media lies in the number of parents used in a vial. It is generally best to use from 5 to 10 pairs of parents in starting a new culture. With smaller numbers there may be too few larvae in the food. Under these conditions a mold

may take over the culture. The occasional moldy vial should be heated to kill the flies and mold and then carefully washed before re-use.

At 72° F. to 77° F. from 10 to 15 days are required for each generation. To maintain stocks of flies it is best to change the adults present to a fresh food vial about every 7 to 10 days. Thus all stages will be on hand at almost any time. When a food vial has been in use for about 3 weeks it should be placed in an oven at about 225° F. for about one-half hour and then washed for re-use.

Cotton plugs may be used several times if they are clean and free from mold. It often helps to heat them in an oven at 200° F. for an hour as a sterilization procedure. This is not essential if ordinary care is used in dealing with molds.

In a subsequent article, a classroom experiment in natural selection, or evolution, will be described. It is much simplified and brought within the scope of the elementary biology class by use of the methods presented here.

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Arnold, Lloyd L., II, 1957. Breeding Drosophila in disposable paper containers. *American Biology Teacher.* 19 (8): 248-251.

BIOLOGY IN THE NEWS

BROTHER H. CHARLES, F.S.C.

TWILIGHT OF THE GREAT BEASTS, Smith Hempstone, *Saturday Evening Post*, November 19, 1960, pp. 21-23, 72-77.

Lions, elephants, in fact all the large animals of Africa are being crowded out of their range by the increasing use of the land. What is a sane policy relative to their preservation? All students should read this article.

THE BOUNTY HOAX, Ben East, *Outdoor Life*, December 1960, pp. 29-31, 81-85.

Do bounties control predators, or are the millions of dollars paid in bounties wasted? What do hunters and conservationists think of the bounty system, and why? This article presents some interesting facts and views.

LIFE'S MYSTERIOUS CLOCKS. Frank A. Brown, Jr., *Saturday Evening Post*, December 24, 1960, pp. 18-19, 43-44.

Nature's timing systems regulate the behavior of all forms of life. But how? Are the activities of plants and animals regulated by some external forces or do organisms have built-in clocks? Dr. Brown suggests some answers.

PURSUIT OF A PAINKILLER, Ben Pearse, *Saturday Evening Post*, December 24, 1960, pp. 16, 88-89.

The story behind the long and difficult development of a synthetic narcotic to replace the useful but addictive drug, morphine.

HOW GOOD ARE 900-CALORIE LIQUID DIETS? Wallace Croatman, *Redbook*, January 1961, pp. 36-37, 93-94.

Many overweight Americans are using low calorie liquid diets. This short article can stimulate lively discussion from both physical and psychic angles.

Physical Facilities

If laboratory experience is the best way for a child to learn science—and most teachers believe it is—the lack of school laboratories and other physical facilities can put a real crimp in the current lively efforts on several fronts to improve the science curriculum.

So says the National Science Teachers Association (NSTA) in launching a major national Science Facilities Study. Its purpose: to determine the physical setting and tools which must undergird a top-notch school science program all the way from kindergarten through junior college.

The NSTA Committee will study physical facilities along with other related questions having to do with expected future enrollments in science: the coming supply of qualified science teachers, new teaching methods, and aids which can be used in the service of science, such as closed-circuit television, microfilm resources, and teaching machines.

Genetics Experiments in High School Biology

HAROLD L. EDDLEMAN
Salem-Washington Township High School, Salem, Indiana

Most biology students finish the study of inheritance wishing that they could duplicate some of Mendel's experiments. Fruit flies having a life cycle of 10 to 15 days and flour beetles having a life cycle of three weeks have proven useful for genetics experiments at the high school level. Fruit flies have four pairs of chromosomes and flour beetles have ten pairs. In both insects, sex-inheritance is of the XY type with the XY individuals being males as in the case of man.

Both insects are reared on simple foods available from the grocery store. The techniques for handling the insects have been simplified so that any student may be expected to obtain results. We rear the insects in ¾ oz. coffee creamers. Since twenty-four creamers will fit into a cigar box, space requirements are modest and each student may perform one or more crosses.

About two hours of class time is required for the experiment, and it appears to be time well spent for misinterpretations that have survived class discussion are often clarified by the experiments.

We begin the experiments during the two-week unit on genetics by mating the parents (P_1). The parents are removed before the offspring emerge from the pupal stage. Later the offspring, F_1, are classified according to phenotype and the results recorded. Some of the F_1 individuals are then placed in fresh cultures to give an F_2 generation which will demonstrate Mendel's Law of Segregation.

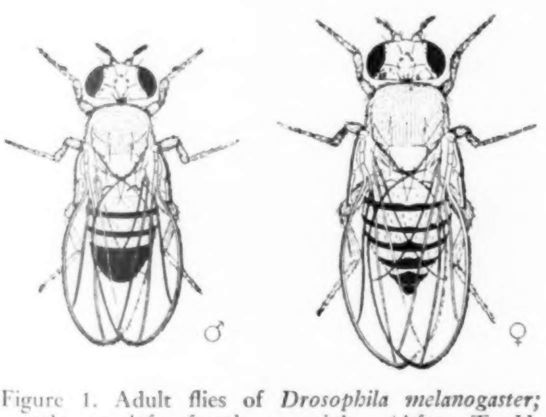

Figure 1. Adult flies of *Drosophila melanogaster;* male on left, female on right. (After T. H. Morgan.) From *Drosophila Guide*, Carnegie Institution.

The ratios seldom come out exactly as expected, and this presents an opportunity to mention statistical tests in the analysis of data. When the results of individual students are tabulated on the blackboard and totaled, the sum is usually near the expected ratio, illustrating the fact that ratios are more accurate when working with large numbers of organisms.

A Genetics Unknown*

The experiment is presented as an unknown. This is a novel approach for most students, and it generates enthusiasm. Each student must study the inheritance of an assigned mutant, which is identified only by a number, and write a report to include 1) a description of the mutant, and, 2) answers and supporting data for the following questions:

1. Is the trait inherited?
2. If so, how is it inherited—as a single gene, two pairs of interacting genes, or quantitatively (the additive effect of many genes)?
3. Does the gene have secondary effects? Are these harmful or beneficial? Many genes have manifold effects; e. g., in humans, sex determines the pitch of voice and growth of beard.
4. Is the gene dominant? Recessive? Or lacking dominance?
5. Is the trait sex-linked, Y-borne, sex-limited, sex-influenced, or autosomal?

The better students are encouraged to pursue the following additional questions for extra credit and/or extra-class projects.

6. On which chromosome is the gene located?
7. Where on that chromosome? A. genetically? B. cytologically?

*These experiments based on suggestions by Taylor Hinton (5).

Procedure for Unknown

The following cross will answer questions 1, 2, 3, 4, and 5:

$$P_1 \quad \frac{\text{mutant}}{\text{mutant}} \text{ virgin } ♀ \quad \times \quad \frac{\text{wild } (+)}{\text{wild } (+)} ♂$$

$$F_1 \quad \frac{\text{mutant}}{\text{wild}} \text{ (inbreed these)}$$

If the mutant is sex-linked all F_1 males will show the mutant trait.

F_2 Classify 100 organisms to obtain ratio of mutant to wild type.

To locate the gene on the proper chromosome, if working with Drosophila (question 6):

$$P_1 \quad \frac{\text{mutant}}{\text{mutant}} ♀ \quad \times \quad \frac{Cy\ +}{+\ Pm}; \ \frac{H\ +}{+\ Sb} ♂$$

F_1 If sex-linked, all males will be mutant type. If not sex-linked, expected types are:

Cy; H Cy; Sb Pm; H Pm; Sb	The mutant unknown will not appear in F_1 if it is recessive.

Select males of one of the four types for the next cross. Use the type which is most easily studied in combination with the particular mutant unknown; e. g., curly wing, Cy, would not be used with a wing mutant.

Mating to obtain F_2: $\frac{\text{mutant}}{\text{mutant}} ♀ \times$ Cy; Sb ♂

(or one of the other types chosen)

F_2: If mutant unknown does not appear in any fly carrying the second chromosome markers (Cy or Pm), the unknown is located on the second chromosome.

Similarly, if the mutant unknown is located on the third chromosome it will not appear in any fly carrying the third chromosome markers (H or Sb).

If the unknown mutant appears in flies also carrying second and third chromosome markers, we know that the mutant is on the fourth chromosome since the possibility of its being on the first pair (X or Y) was eliminated by the first experiment.

To determine the location of a gene on a chromosome (question 7), cross the mutant with a stock carrying several mutants on that chromosome and back cross F_1 females with males of the marked stock. The percentage of crossing over will indicate the location of the unknown mutant on the chromosome.

Determination of the cytological location of the mutant gene involves examination of salivary chromosomes (1, 2, 3).

A few of our students have succeeded in answering question 7A. Although none has answered 7B, several have learned much about staining techniques by attempting the procedure.

Culture of Fruit Flies (Drosophila)

Detailed instructions for the culture of *Drosophila* are given in the *Drosophila Guide*, a 44-page booklet by Demerec and Kaufmann (3). Single copies are free to teachers and additional copies are only twenty-five cents each. The guide gives detailed instructions on media, hybrid crosses, linkage tests, preparation of salivary gland chromosomes, and other interesting topics. Any teacher may obtain free cultures of the *Drosophila* mutants needed for the experiments in the guide by writing to the Department of Genetics, The Carnegie Institution of Washington, Cold Spring Harbor, New York.

Fruit flies live on growing yeast cells and will thrive on a variety of carbohydrate-rich media that have been inoculated with ordinary baking yeast. Overripe bananas dipped in yeast will rear large numbers of flies, but the fruit becomes liquid making it difficult to remove the flies. A medium which will remain firm is preferred because flies are generally collected by inverting the bottle and shaking the flies into an etherizer. Oatmeal, cream of wheat, or agar will produce solid medium. A mold inhibitor, such as Moldex supplied by the General Biological

Supply House, is generally used; but a strong growth of yeast is, perhaps, the best mold inhibitor.

We use the following medium:

Part A
- Water—1 liter
- Agar—8 grams
- Moldex—0.7 grams (10 ml. of a 0.07 gm/ml tincture)

Part B
- Molasses—200 grams
- Corn meal—100 grams

Mix the ingredients of part A and boil gently for five minutes to dissolve the agar. Combine the corn meal and the molasses (we use brown Karo), mix to a smooth batter, and then pour into part A while stirring. Boil gently about ten minutes. Pour to a depth of one-half inch in coffee creamers or milk bottles and cover with paper towels to prevent mold contamination. Cool overnight to allow evaporation of excessive moisture. Now, sprinkle a few particles of dry yeast into the creamer, insert a piece of paper towel for flies to rest on, and stopper with a cardboard cap or cotton plug. Store at room temperature until needed; do not refrigerate. Bottles will keep about one week.

Drosophila should be reared within a temperature range of 20° to 25° C. Temperatures above 30° C. will sterilize fruit flies. They should have eight hours or more of light each day.

In making crosses, virgin females must be used. Since *Drosophila* females do not mate until they are about twelve hours old, the adults are shaken out at intervals of less than 12 hours, etherized, and sexed. The sexes are segregated and held until needed in bottles containing food. Adults can be held one to six weeks, but there may be considerable mortality caused by flies becoming mired in the moist food.

The sex of fruit flies is determined by observing the shape of the abdomen. The abdomen of the female is slightly longer and somewhat more pointed at the tip than that of the male. Also the abdomen of the female bears seven dark bands while only three to five bands are visible on males. Males also bear sex combs consisting of ten bristles on the distal surface of the upper tarsal joint of the front pair of legs. Most mutants can be sexed without the aid of a microscope.

List of Easily Classified Drosophila Mutants

Name of mutant	*chromosome*	*symbol***	*locus on chromosome*
yellow body	X	y	0.0
white eyes	X	w	1.5
vermillion (eyes)	X	v	33.0
bar (eyes)	X	B	57.0
dumpy (wings)	2	dp	3.0
black (body)	2	b	48.5
vestigial (wings)	2	vg	67.0
sepia (eyes)	3	se	26.0
ebony (body)	3	e	70.7
eyeless	4	ey	0.2

***Drosophila* and *Tribolium* geneticists use uncapitalized symbols to represent traits recessive to the wild-type trait and capitalized symbols for dominant traits. A plus-sign (+) is used to represent all wild-type traits.

The Culture of Flour Beetles (Tribolium)

We prefer to use flour beetles in genetics experiments because they are hardier, and it is more convenient to obtain large numbers of virgin females. *Tribolium castaneum*, the rust-red flour beetle, is a common pest in stored cereal grain products. The adults are 3.5 mm. in length. Females lay 10 to 15 eggs per day. The eggs measure .60 mm. by .35 mm. The larvae are whitish and molt an average of seven or eight times. Mature larvae are 6 to 7 mm. long.

Flour beetles do not require light at any

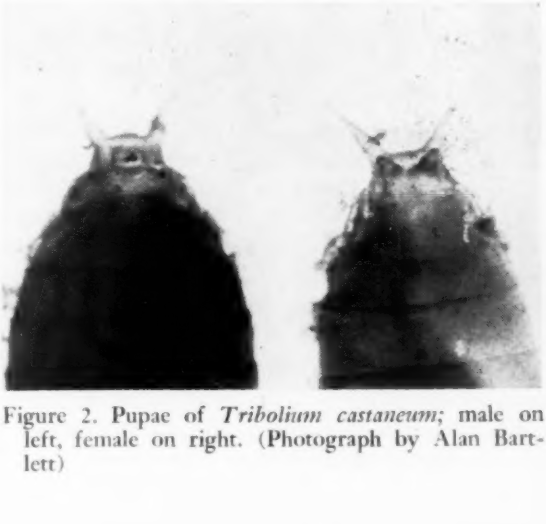

Figure 2. Pupae of *Tribolium castaneum;* male on left, female on right. (Photograph by Alan Bartlett)

Figure 3. Kenny Mitchell prepares to mate females of a mutant strain of flour beetles with wild-type males.

stage of growth, and they live about two months. Stock cultures containing 50 gm. of medium should be transferred every two months, but larger cultures may survive a year at room temperature making it possible to maintain genetic stocks in the laboratory with only infrequent attention. It is wise to carry more than one culture of each mutant to avoid losing a stock.

Flour beetles will reproduce within a temperature range of 70° to 98° F., with the relative humidity within a range of 10 to 75%. The length of the life cycle at 70° F. is about seven weeks, at 90° F. four weeks is required, and at 98° F. only eighteen days is required. Since there is some reduction in the number of offspring at 98° F., the optimum conditions appear to be 90° to 95° F. with the relative humidity between 50 and 70%. We use an old chick incubator to maintain these conditions.

Tribolium are reared on a dry mixture of 95 parts sifted whole wheat flour and 5 parts of dry brewer's or baking yeast. Bisquick and poultry mash are other suitable foods. The medium is dry-sterilized at 60° C. for 6 to 8 hours to destroy the eggs of wild beetles and parasites. The sterile medium may be stored indefinitely in insect-tight containers. The nutritive factors appear to be stable over a period of one or more years.

Coffee creamers, small jars, and milk bottles are suitable culture containers. Commercial cardboard caps are used on the creamers and milk bottles. The insects obtain sufficient air through the cardboard without holes.

Tea saucers and syracuse staining dishes are useful for examining the beetles since they do not fly and cannot climb clean porcelain or glass surfaces. Bits of cardboard, wooden splints, or camel hair brushes (sizes 1, 2, or 3) are handy for manipulating the beetles.

Adult beetles, pupae, and large larvae are conveniently separated from the medium by sifting. Sieves having 30 to 40 meshes per inch are most convenient. In preparing the medium, the whole wheat flour is sifted through a sieve having slightly smaller pores to eliminate the large bran particles which would otherwise be collected on the sieve with the beetles. Cast larval skins and other debris are easily separated from the beetles by blowing gently upon them.

A cheap, durable sieve can be made by placing a square of brass strainer cloth on a hot stove and pressing against it a 1¼ inch section of 1½ inch or larger diameter black plastic pipe. The plastic will melt into the pores of the cloth making a secure joint. Trim the excess cloth and smooth the edges on an emery wheel.

Fifteen x is a convenient magnification for determination of sex and eye colors while lower powers are more satisfactory for determination of other traits. Of the twenty or so known mutants in *T. castaneum*, approximately half can be distinguished by the naked eye or with the aid of a 4x hand lens.

The sexes in *T. castaneum* are mostly easily

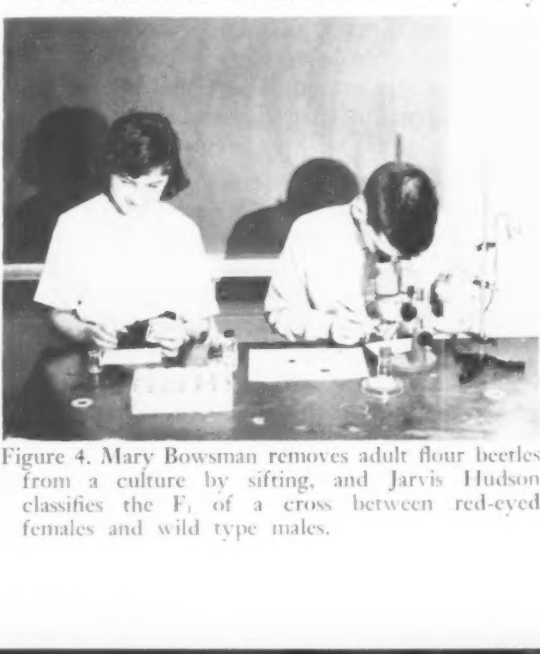

Figure 4. Mary Bowsman removes adult flour beetles from a culture by sifting, and Jarvis Hudson classifies the F_1 of a cross between red-eyed females and wild type males.

distinguished during the pupal stage which lasts 5 to 7 days. One doesn't have to visit the lab in the middle of the night or during weekends to obtain virgin females as is often necessary when using *Drosophila*. Examine the ventral surface of the pupa under a magnification of 15. At the caudal end of both sexes there is a forked structure, but just anterior to this one finds in the females another somewhat smaller forked appendage. This appendage is greatly reduced in the males. See Figure 2. Adult males can be distinguished by a small raised "sex spot" on the medial surface of the femur. The sex spot is more easily seen in young males since it becomes pigmented at a faster rate than the remainder of the body.

Etherization of Insects

Since fruit flies are active flying insects it is necessary to etherize them for examination. Although flour beetles do not fly, they must generally be etherized for examination of eye color and other minute details. Either insect may be etherized in a creamer or milk bottle which has a bit of ether-moistened cotton wired to the stopper. Etherize insects just enough to immobilize them because overexposure may cause sterility or death.

An etherizer requiring only a few drops of ether per hour may be constructed by placing a bit of cotton in the bottom of a creamer and inserting a perforated No. 10 gelatin capsule wrapped with a bit of paper for a snug fit in the neck of the creamer. After moistening the cotton with ether, pour insects into the capsule and replace the cap

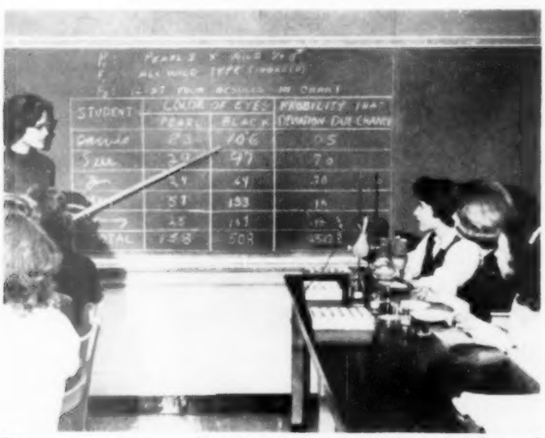

Figure 5. Donna Ratts summarizes the results of five students assigned the pearl mutant as genetics unknowns.

Figure 6. Monty Williams inspects *Tribolium* cultures in an incubator that he built for experiments in his home laboratory. A light bulb (no thermostat) and a pan of water maintain temperature and humidity.

on the capsule until the insects are sufficiently immobilized.

List of Viable Tribolium Mutants*

Sex-linked:

pd: paddle. Distal segments of antennae fused to give a paddle shape. Recessive.

py: pygmy. Body two-thirds normal length. Recessive.

r: red. Eye color pink to cherry-red depending upon age. Recessive.

Mutants located on autosomes:

b: black. Black body and appendages. Incompletely recessive.

c: chestnut. Eye color chestnut brown. Recessive.

d: dusky. Body color black, but appendages lighter. Recessive.

p: pearl. Eye pale gray. Recessive.

p^{pk}: pink. Eye pink. Allelic to pearl and dominant over pearl, but recessive to wild type eye color.

*Any five of these mutant types and a wild type are available for one dollar from the Lyon Science Club, Salem High School, Salem, Indiana.

s: sooty. Very dark brown body color. Recessive.

sq: squint. Eyes squinted. Probably blind. Recessive.

barbed. Enlargement of structure above eye gives head a barbed appearance. Symbol and inheritance not known to writer at this time.

Getting Started

The day of the experiment clearly marked saucers holding virgin mutant females and wild type males are placed in the classroom. Fruit flies are etherized but flour beetles are not. Each student picks up a creamer and brushes two females and two males into it. If fruit flies are being used, the creamers have been prepared before class; but when working with flour beetles the student fills the creamer to a depth of three-eighths of an inch with sterile medium. After labeling the culture with the symbols of the parents mated, the date, and his initials, each student gives his culture to his section leader. The section leaders are students who assist the instructor by operating the incubator and inspecting the cultures regularly.

The section leaders also supervise the removal of the parents, counting and inbreeding of the F_1 generation, and the counting of the F_2 generation. Each of these operations requires little time and seldom interferes with the work of later units. Students from previous years who have continued their work in genetics on an individual project basis have creamers, virgin females, and other supplies ready for use in return for the privilege of using school equipment in their projects.

Statistical Evaluation of Results

To obtain full credit the student must evaluate his results using the chi-square method. This seems desirable since biology courses are notoriously devoid of the mathematical applications used by the research biologist.

Chi-square, X^2, is determined by the formula, $X^2 = \Sigma\ (d^2/e)$. The letter, d, represents the deviation (difference) of the observed number, a, from the expected number. The expected number, e, for a given class is found by multiplying the total number of offspring by the proportion expected in that class. Thus, chi-square is found by calculating d^2/e for each class of offspring and adding all the quotients together.

Students understand the calculations more readily when they are presented in table form, as in the problem below: "a student mated pearl-eyed female beetles with the wild type males and obtained in the F_2 generation 115 offspring with wild type eyes and 39 with pearl eyes. What is the probability that these deviations from a 3:1 ratio are insignificant, random, sampling errors?"

Table of Calculations

Class	*Number observed (a)*	*Number expected (e)*	*Deviation (d) (a - e)*	$\frac{d^2}{e}$
Pearl	39	38.5	0.5	.006
Wild	115	115.5	0.5	.002
Total	154	154.0	—	.008

To convert our value for chi-square, .008, to a probability we must consult a table of chi-squares. Since we have two classes of offspring and the number of degrees of freedom is usually one fewer than the number of classes, we must locate our value of .008 in the line corresponding to one degree of freedom. We obtain $P = .98$. This means that a deviation of the magnitude represented by our data can be expected in 98% of the cases, and it indicates that our deviation was probably just a sampling error.

Should P be some value less than .05 we would suspect that some factor other than chance sampling error was responsible. Some causes of significant deviations from the expected ratio are interaction of genes, high mortality of the individuals homozygous for the mutant gene, or the use of too few progeny.

Suggested Projects

As for other units, we suggest some possibilities for individual research. Some that our students have pursued with success are:

1. Preparation of chromosome maps by studying the crossover percentages between linked genes.
2. Inbreeding of wild fruit flies to determine the number and frequency of mutant genes in natural populations.
3. Inbreeding corn to obtain lines having widely differing characteristics.

4. A Tribolium research team which has found several new mutants in x-irradiated stocks (4).

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Atomic Energy Commission's Report

The report summarizes the Commission's Isotopes Development Program which is aimed toward accelerating the peaceful uses of radiation and reactor byproducts. It cites some of the major milestones in isotopes development; the uses of radioisotopes and radiation in agriculture and plant studies; the impact of radioisotopes on medical diagnosis and therapy; the utilization of radioisotopes in physical and chemical research; the impact of radioisotopes on life science research; the development of new applications for using radioisotopes; applications for radiation; training programs regarding utilization of radioisotopes; the radioisotope production program; and safety programs for radioisotope utilization.

The appendices include data on the distribution of radioactive isotopes; research contracts let to further radioisotope utilization; a film list; and the Commission regulations relating to radiation and radioisotope usage.

Copies of "Radioisotopes in Science and Industry" may be obtained for $1.25 from the Superintendent of Documents, Government Printing Office, Washington 25, D. C.

Teachers Wanted Abroad

Interested in teaching overseas? A number of openings for teachers under the Point Four program are available in Asia and Africa with International Voluntary Services, a nonprofit organization that sends technical assistance teams abroad.

A team of teachers will be working in rural areas and villages of a small country in West Africa. Members of this team will teach at the elementary level, help direct 4-H type programs, and lead in community improvement projects.

Each two-year contract provides transportation to and from the project, all housing, food, clothing, and vacation allowances, insurances—in short, all necessary living expenses—plus a reasonable cash salary. Deferment from military service may be secured.

Candidates must be single men who have had some teaching experience. Preference will be given to those with a rural background or agricultural education.

During 1961, openings will also be available in South East Asia for those qualified to teach English in non-English speaking schools on both elementary and secondary levels. These posts will be open to both single men and women.

Applications and further information regarding teaching positions can be secured by writing to International Voluntary Services, Inc., 3636 16th Street, N.W., Washington 10, D. C.

NESSAC

A new organization, called NESSAC, the New England School Science Advisory Council, has been formed in that area. It publishes a Newsletter which indicates the great many services which New England industry is ready to perform for school systems in that area. Dr. Dorothy Ladd, MIT Instrumentation Laboratory, 68 Albany Street, Cambridge, Massachusetts, is Editor.

Radiation Studies with Neurospora crassa

JAMES D. REGAN, *University of Hawaii, Honolulu 14*

In many high schools and junior colleges adequate facilities are not available for the study of the effects of radiation on biological material. However, certain aspects of radiation biology, for example, lethality and photoreactivation, can be demonstrated utilizing the effect of the ultraviolet radiation from a germicidal lamp upon the cells of the ascomycete, *Neurospora crassa*, the well-known genetics organism.

The materials required are: petri plates with covers, pipettes, bottles of dilutions, bent glass rods for spreading conidia on plates, transferring loops, cotton for plugging, Difco *Neurospora* culture agar, and a pressure cooker for sterilizations if no autoclave is available. Ultraviolet germicidal lamps are usually obtainable at the drug store at a cost of five to seven dollars. A culture of strain 70007-A (a morphologically colonial strain) of *Neurospora crassa* (ATCC catalog #13792) may be obtained from the American Type Culture Collection, 2112 M Street, N.W., Washington 7, D. C. Cultures are five dollars each to educational institutions.

Upon receiving the culture, the instructor should prepare several 125 ml., or larger, erlenmeyer flasks with 3/8 inch of *Neurospora* culture agar, prepared as per directions on the bottle. The flasks are then plugged with cotton and sterilized, after which they may be inoculated from the original culture by means of a transferring loop. Sterile technique should be used throughout; i.e., flaming mouth

Figure 2. Conidial suspension is filtered through sterile cheesecloth to remove clumps and hyphae.

of flask, transferring loop, etc. The conidia usually require five to six days for maximal production. They are harvested by scraping them from the surface of the agar with a flamed loop and transferring them to a sterile bottle containing 75 ml. of sterile water. When enough conidia have been transferred to the bottle of water to make the suspension quite turbid, it is poured through sterile gauze into a sterile flask to remove clumps and hyphae. Aliquots of 10 ml. are then pipetted into five, numbered petri plates without agar for irradiation. Plate #1, the control, receives no irradiation. The other four receive irradiation doses of 30, 60, 90, and 120 seconds at a distance of about 20 cm. from the UV lamp. The instructor should run a preliminary irradiation experiment to determine what exposure intervals will give

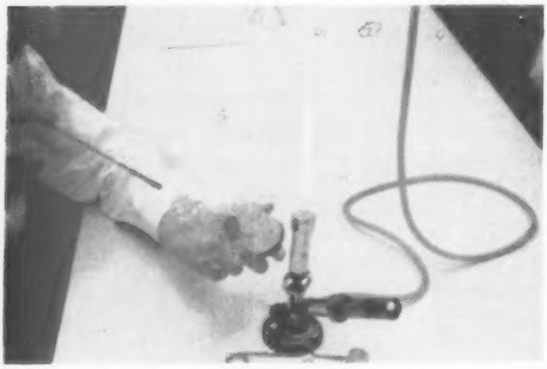

Figure 1. Conidia are harvested from culture and placed in a dilution bottle using transferring loop.

Figure 3. Aliquots of conidial suspension are pipetted into numbered petri plates for irradiation.

Figure 4. Aliquots of the irradiated and diluted conidial suspension are pipetted onto agar plates.

a good kill curve; the above intervals are suggested as a start, but these may have to be lengthened or shortened depending on the output of the lamp. The conidia are irradiated in open petri plates and then covered and kept in the dark for one hour to prevent photoreactivation. Each of the aliquots of conidial suspension including the control are then diluted to dilution intervals of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. 1 ml. of original suspension into 99 ml. of sterile water equals 10^{-2}. Since over- or under-populated plates cannot be counted conveniently, these dilutions must be made in order to get two or three sets of plates that have adequate numbers of colonies but are not so over-populated they cannot be counted at all.

Petri plates for growing the irradiated conidia are prepared by pouring sterile *Neurospora* culture agar, cooled to 45° C., into sterile plates to a depth of about ¼ in. These should be covered immediately and allowed to cool. Plating is accomplished by

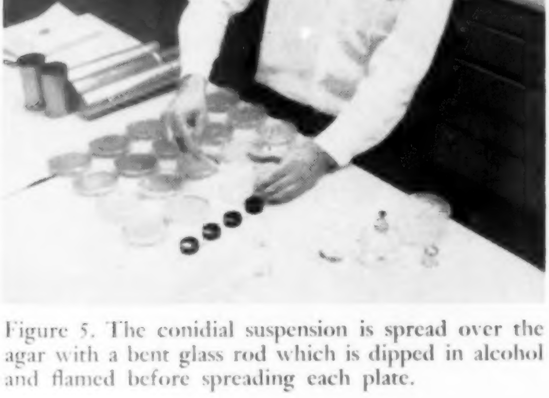

Figure 5. The conidial suspension is spread over the agar with a bent glass rod which is dipped in alcohol and flamed before spreading each plate.

Figure 6. Colonies are counted and tabulated.

pipetting 0.2 ml. of the diluted conidia onto the agar plates and spreading with a flamed glass rod. Five plates per dilution interval should be inoculated, since usually only two or three sets of dilution plates will be countable.

After three days incubation at room temperature, the colonies should be counted and tabulated as the number of surviving colonies at each dose. The data may then be plotted as per cent survival vs. dose. This method will give a kill curve which will show graphically the relationship between these two variables.

For photoreactivation studies, a duplicate series is prepared exactly as outlined above. After irradiation, however, the conidia are subjected to bright white light for one hour instead of being kept in the dark. Thereafter they are treated the same as the above series. The photoreactivation effect may be seen by comparing the survival data for the two series.

Should the instructor desire to do some reading on the phenomenon of photoreactivation, an excellent review by Jagger may be found in *Bacteriological Reviews*, Volume 22, No. 2, pp. 99-142, 1958.

AIBS Meeting

The NABT will meet with the AIBS in its annual meeting at Purdue University, Lafayette, Indiana, the last week in August.

Seed Extracts and Human Blood Typing

JOHN GABRIEL NAVARRA and LILLIAN KATZ
Jersey City State College, Jersey City, New Jersey

The study of blood groups has become a normal part of the biology course at both the high school and college level. In fact, students may type their own blood as a normal part of the laboratory work.

Laboratory work of this nature can become part of a larger study of antigen-antibody combinations and reactions. The suggestion which follows is for a laboratory technique which demonstrates that hemagglutinating substances occur in the seeds of certain plants. Extracts of certain seeds can be prepared by students. These seed extracts can then be used to produce the agglutination of human blood cells.

Performing The Experiment

Materials Needed:

1) Blood from group A, group B, and group O individuals.
2) Standard commercial preparations of anti-A and anti-B human blood grouping serum.
3) An assortment of different kinds of lima beans bought at any food store.
4) A mortar and pestle or a hand pepper mill for grinding the seeds.
5) Glass well slides and standard chemical test tubes
6) A standard laboratory centrifuge.
7) Several hundred milliliters of normal saline. Normal saline is made by preparing a 0.9 per cent solution of sodium chloride. Dissolve 0.9 grams of NaCl in 100 ml. of distilled water.

Methods To Be Used:

1) *Preparation of the seed extracts:*

Grind the seeds in the pepper mill or with the mortar and pestle until a meal of the consistency of coffee ground for a perculator is obtained. Add to the meal ten times its weight of normal saline solution. Amounts of 15 gms. of seed plus 5 ml. of saline solution will provide enough of the required 10% extract for testing.

Agitate the mixture thoroughly. This may be accomplished by shaking the mixture vigorously for a period of a minute. Repeat the vigorous agitation at ten minute intervals. Agitate the mixture at least six times over a period of one hour. When the agitation of the mixture has been completed, centrifuge the mixture at 3,000 r.p.m. for five minutes. Discard the sediment and retain the supernatant fluid which contains the extract.

2) *Preparation of blood suspensions for testing:*

Add about 60 drops of normal saline to a test tube. Puncture a donor's finger with a lancet or sharp needle that has been sterilized with heat or 70% alcohol. Squeeze the blood, drop by drop, from the punctured finger into the saline solution. Ten drops of blood should be sufficient.

The mixture of blood and saline should be centrifuged. The supernatant saline is then discarded. You will now have packed red cells at the bottom of the centrifuge tube.

Place 0.2 ml. of the packed red cells in 10 ml. of normal saline. This gives you approximately a 2% suspension of red cells.

3) *Performance of the tests:*

a) *Determining the blood type of the suspension:*

Place a drop of the 2% suspension of blood in the well slide. Add one drop of anti-A serum to the suspension of the blood. On another well slide, mix a drop of anti-B serum with a drop of the blood suspension. Allow the slides to stand at room temperature and then gently agitate the slides

Possible Results:

Examine the illustration. The blood suspension will be one of the four types: A, B, AB, O. There will be no agglutination if the blood is type O. There will

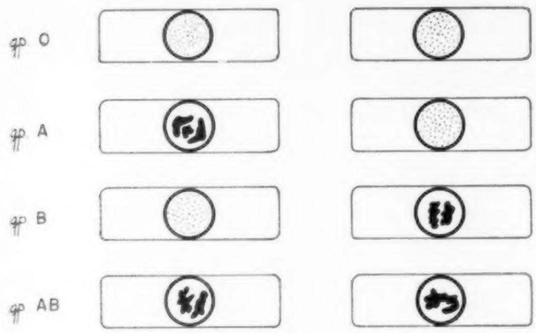

ILLUSTRATION *1

be agglutination with both anti-A and anti-B if the type is AB. Type A blood will only agglutinate with the anti-A, and agglutination will only occur with anti-B in the case of a type B blood.

b) *Agglutination of human blood cells by the seed extract:*

Mix a drop of the 2% blood suspension with a drop of the seed extract. Allow the slide to stand at room temperature for ten minutes and then gently agitate the slide. A positive reaction will be indicated by the presence of clumps, or masses of agglutinated cells with a clear background, on the well slide. A negative reaction will show only a smooth mixture of cells in saline.

Possible Results:

With the lima bean extract agglutination of group A blood will occur. There should be no agglutination of either group B blood cells or group O blood cells. However, some bean extracts may prove to be nonspecific. They will give a weak reaction with either the group B or the group O blood in addition to a strong reaction with the group A blood. If you should have AB blood the lima bean extract will, of course, produce a strong reaction. A strong reaction will produce large clumps of blood which are very difficult to break up; a weak reaction will produce smaller clumps more evenly dispersed over the entire well.

Implications Of The Experiment

Tests may be made with extracts of the seeds of a number of very common plants. The extract of a number of these common plants is specific for blood group A. Their routine use for blood typing on a large scale is, at this time, not very practical, since no plants have as yet been found which will yield an agglutination specific for blood group B, or for the varieties of Rh positive blood

However, since the cost of such preparations would be low, and the source extremely abundant, this is potentially of great practical importance. Also, the existence of chemical compounds in plants so similar to those found normally in humans has many important implications in regard to basic biochemical research.

Groups of students may be encouraged to undertake this type of experimentation to catalogue types of reactions with extracts of many types of seeds.

Science Fair Winners

Biological exhibits prepared by teen-aged finalists in the 1960 National Science Fair-International will be exhibited at the All-India Science Congress at the University of Roorkee, India, January 2 to 9.

The American Institute of Biological Sciences announced in Washington that the two winning displays will form an important part of its large exhibit on biological science at the Indian Congress.

The student exhibits previously had been displayed at the Institute-sponsored Annual Meeetings of Biological Societies in Stillwater, Oklahoma, attended by 2,500 American scientists.

The exhibits were those entered by Susan Brown, 2604 Pecos Street, Austin, Texas, a graduate of Stephen F. High School, who now is attending the University of Texas, and Gary Botting, 314 Pearl Avenue, Peterborough, Ontario, Canada, a student at the Peterborough Collegiate and Vocational School.

Susan's project resulted in isolation of a root growth factor from pinto bean seedlings. Gary's display demonstrated his knowledge of Cynthia moths.

Each display won honors among biological exhibits in local Science Fairs. The two were outstanding among those entered in the National Science Fair-International at Indianapolis, Indiana, conducted by Science Service.

Demonstrating Sap-Rise

REV. ANSELM M. KEEFE, O. PRAEM.
St. Norbert College, West DePere, Wisconsin

The use of white celery stalks immersed in laboratory dye solutions to demonstrate the speed of sap rise in plant petioles has long been a stock experiment in botany laboratory manuals. Occasionally one hears complaints that the experiment failed or at least left much to be desired. Investigation generally reveals that the failure is due either to the type of dye solution used or to faulty techniques in handling the living materials.

As far as the dye solution is concerned, none of the reagent dyes usually suggested in laboratory manuals is half as effective as the ordinary kitchen variety of food coloring solutions. Several kinds of these are sold in almost every grocery store. They are always nontoxic and edible because their main use is for coloring candies, cake frostings, and the like. The red coloring solutions have been found most effective because of the contrast they establish with the more or less greenish leaflets of the celery. The accompanying illustration shows several stalks so treated in comparison with an untreated control.

The second cause of failure may be due to not reestablishing the continuity of sap movement upward in the celery petioles.

Celery stalks may be used in normal condition just as they are bought in the food stores, but the sap rise will be correspondingly slower than in plasmolyzed material. It is in the rapid reestablishment of the sap stream and in the restoration of turgidity that the success of this demonstration lies. The fact that so many unscientific means are used by those who wish to resuscitate wilted cut flowers makes one suspicious that this phase of plant physiology is pretty much taken for granted in high school and elementary college botany courses.

Celery may be wilted by leaving it out of water and uncovered overnight in a warm room or in a current of air. Best results will be obtained if it is in a distinctly floppy condition when used. The secret of success is to cut off an inch or two from the bottom of each stalk with a sharp knife *under water.* This eliminates the air blocks which have been sucked into the xylem strands as the upper leaves of the stalk evaporated their water content.

The cut end of the stalks are swiftly transferred from the cutting dish to the dye solution. Here it is important that the water film across the cut end of the stalk is not shaken off. The dye movement starts immediately. With a sweep-hand watch the seconds can be counted until the first noticeable flush of the dye appears near the axils of the leaflets.

After this part of the demonstration is completed the stalks may be sliced across and the sections distributed to the class for observation or the dissection out of the colored vascular bundles for further study.

Those who teach botany in night schools, or who have laboratories which may be thoroughly darkened, may wish to use comparatively colorless 10% solutions of fluorescein or esculin instead of the dye solutions. The demonstration must be performed under "black light" or ultraviolet radiation with the room lights off. The rise of the soluble fluorescent materials in the plant petioles gives results which are little short of spectacular.

In schools where a Geiger counter, or preferably a scaler-counter, is available, a more

spectacular demonstration is possible. A capsule of the radioactive isotope Iodine 131 is dissolved in 100 ml. of distilled water with or without the dyes just mentioned. Two types of these capsules containing 25 or 100 millicuries of I^{131} are available at hospitals where radioactive isotopes are used for diagnostic or therapeutic purposes. Those of low intensity do not require an Atomic Energy Commission license for their use. The procedure for preparing the celery leaves is as outlined above. The probe of the counter is placed behind the topmost leaflets. Within a few minutes the counter picks up more than the cosmic background discharges. As the iodine solution accumulates in the leaflets the speed of the radioactive discharge increases to a most satisfactory degree.

Air Force Overseas Dependent School Program 1961-1962

The Air Force Overseas Placement Officer located at Civilian Personnel Office, Selfridge Air Force Base, Michigan, is accepting applications and interviewing educational personnel for Dependents' Schools overseas for the 1961-1962 school year. The purpose of these schools is to provide stateside public school educational opportunities, grades 1 through 12, to dependents of military and civilian personnel stationed overseas. Approximately 75% of the position vacancies will be in the elementary grades.

The locations will be: Azores, Bermuda, Crete, Denmark, England, France, Holland, Germany, Iceland, Italy, Japan, Labrador, Libya, Morocco, Newfoundland, Norway, Philippine Islands, Spain, Saudi Arabia, Scotland and Turkey. Vacancies in Crete, Denmark, Holland, Italy, Norway and Scotland are filled from waiting lists of currently employed Air Force teachers.

The tour of duty will be twelve months (normal school year approximately ten months plus travel to and from the overseas area of assignment). Some administrative personnel will serve on a calendar year basis.

Room for Young Scientists

Possibly 40% of the future's scientists are discovering that their world has few roomy corners for the experimenting that is such an important part of becoming a scientist.

This lack of elbow room was evident in a study made of 250 young high school students chosen to attend the National Youth Conference on the Atom in Chicago, October 20-22, 1960. Delegates were selected on the basis of outstanding work at science fairs, through special examinations, and by recommendation of educators.

Administrators of the Conference have told Science Service that about 41% of these teenagers reported that they had too little room to work at home, or no room at all. There was "plenty of room" somewhere in the homes of only eight percent of this group of promising young experimenters.

Their own rooms, the garage, or the attic served as workshops and laboratories for about a third of the students. Eight percent did all of their project work in their high school laboratories, many of which were described as being inadequate.

Eighty percent were responsive to the idea of well equipped community laboratories with space and freedom for work after school and on weekends. They said they would spend six hours a week or more working in such facilities.

Forty percent of the science-oriented students urged better laboratories in their schools and the opportunity to work in them outside of school hours. Many felt a need for professional level books in the school library.

Film Review

Balance in Nature. 1 reel. 16mm sound. 17 minutes. Color $170.00. Educational Collaborators Dr. R. C. Dickson, Dr. William C. Stehr and Dr. Fred S. Truxal. Available from Filmscope, Inc., Box 397, Sierra Madre, California.

The subject covered in this film is illustrated by two life cycles and ecological relationships of aphids and lady bird beetles. The photography is excellent as is the selection of sequences. The film is very good for high school biology and college students taking beginning courses in biology, zoology or entomology. It conveys especially well the idea of predation and checks and balances in a biotic community.

James M. Sanders, *Chicago Teachers College.*

The Use of Simocephalus Vetulus Embryos In the Teaching of Embryology

GENE W. MOSER
Cornell University, Ithaca, New York

The use of the chick embryo is one of the standard animal techniques used in teaching embryology in secondary school biology. This technique is useful in exhibiting the modes of development in vertebrate animals but fails to present to the student experiences in comparative embryology. The chick in its development illustrates a discoidal meroblastic type of cleavage. Supplemental to this type, the student could be shown superficial meroblastic cleavage as is found in the Arthropods.

I would suggest the use of the anomopod embryo as a good example of a centrolecithal egg with superficial segmentation. Representative of the Anomopods are *Daphnia* and *Simocephalus*. The value in the use of these embryos is their availability, size, and facility in classroom microprojection.

Ward and Whipple, *Fresh-Water Biology*, 1918, pp. 676-677, point out that they are easily collected and identified as no microscopic dissection is needed to establish specific characters. Anomopods are found in littoral zones of fresh water environs. Their ability to maintain physiological balance in waters of low oxygen concentration provide a still greater accessibility. When collecting the animals use a glass container since metal containers prove detrimental to their maintenance. They may be kept in aquaria filled with pond water. The animals may serve as indicators of the oxygen concentration in the aquaria. An anomopod kept in a medium of high oxygen concentration will have a pale yellow or white color. If the oxygen concentration in the medium decreases to an oxygen deficient level, the coloration of the body will change to a rose-pink because of an increase in haemoglobin in the animal.

Hoshi, *Science Reports of the Tohuku University*, 4th Series, Vol. 23, No. 1, 1957, p. 42, suggests that no more than ten individuals be kept in a liter of water. If the oxygen concentration becomes deficient, the use of an aerator or the introduction of some aquatic plants will tend to correct the condition. Periodic addition of pond water should be made to maintain a constant water level and provide microscopic food for the daphnids. A pipette should be used to remove any predators which may be in the pond water added to the aquaria.

As anomopods have two methods of reproduction, a few words are needed to explain their use in the teaching of embryology. The summer egg or parthenogenetic form develops in the brood chamber of the adult, whereas the sexual form is released from the adult in the form of an ephippium or egg case. The ephippia take months or even years to develop depending on environmental conditions. However, the parthenogenetic form completes its development and becomes a free-swimmer about forty-eight hours after it is deposited in the brood chamber. The short period of embryonic development of the parthenogenetic form provides an embryo which may be effectively used in classroom demonstration.

I have found that *Simocephalus vetulus* O. F. Muller, will reproduce parthenogenetically by maintaining them in temperatures under 60° F. Normally, sexual reproduction is initiated during the summer months by the temperature and other unknown factors. A *Simocephalus* population maintained in aquaria under the correct temperature will possess individuals with young in their brood chambers, and these young will exhibit various stages of development. Each individual may have in its brood chamber as many as fifteen young, all at a relatively equal level of development. The body of the adult *Simocephalus* is covered with a transparent chitinous carapace. This relative transparency will allow a microscopic examination of the adult to determine the stage of development of the embryos in the brood chamber which is located in the dorsal region of the thorax.

The following procedure is suggested for the display of the embryos. With a pipette, place an adult containing embryos of a desired

stage of development on a depression slide with some pond water. Allow the animal to swim about for a few minutes. With a medicine dropper withdraw enough water to immobilize the animal. Using a size 8 sewing needle or size 0 insect needle, press down the head. With a second needle of the same size, anchor the lower ventral edge of the carapace to the surface of the slide. Using the first needle, lift the upper ventral edge of the carapace, folding it back over the animal. With both flaps of the carapace now held by a pin, gently shake the animal until the young flow out of the brood chamber. When the embryos are free, fill the slide with pond water which has been cleared of microscopic organisms by boiling. If the embryos are to be kept for a long period of time, a culture slide with a concavity 18 mm. in diameter and 0.8 mm. in depth should be used. The embryos may be transferred from the depression slide to the culture slide by a medicine dropper. Fill the culture slide to its brim with cleared pond water and cover the depression with a bottle cap to decrease surface evaporation. In this manner I have kept nine-hour embryos until they developed into free-swimmers about forty hours later.

The photomicrographs accompanying this article illustrate the possible use of *Simocephalus* embryos in microprojection. These photographs are not presented as a description of the development of the animal. Primarily, they are included to illustrate the use of the animal in teaching comparative embryology and the feasibility of employing it in microprojection techniques in the classroom.

All photographs were taken at low power magnification. The embryos were maintained at a pH between 6.0 and 7.0. It seems that this concentration affords the least erratic rate of development in the embryos. All figures except Figure 4 show the dorsal surface of the embryo. Figure 4 is a lateral view.

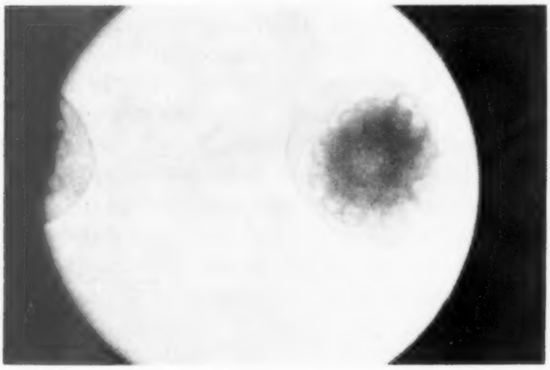

Figure 1

Figure 1 shows the gastrula stage with invagination occurring in the cephalic region. Some limb buds may also be observed in their initial stages of development. The light region in the center of the embryo is a fat deposit. It is believed that this fat deposit contributes to the metabolism of the embryo as the deposit is later observed to disappear.

Figure 2 shows a twenty-seven hour embryo. The brain cell complex is seen in the anterior end of the embryo. The limb bud complexes are more protruded. The fat deposit may be seen in better detail here. Cellular detail is quite distinct in the microprojection of these embryos, as may be noted here. The subsequent development of the sensory system and limb buds would afford the student an experience of showing development which could prove exciting.

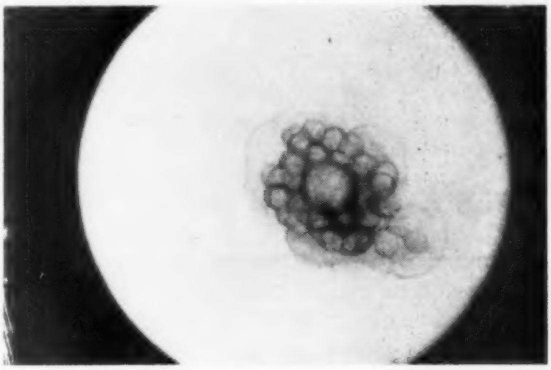

Figure 2

Figure 3 and *Figure 4* show the twenty-eighth hour of development. The caudal region is beginning to show distinct form. The limbs are more discernible, and in the side view their segments may be seen. This stage is the beginning of the period of eye formation. The author has made considerable study of the formation of the two eyes. From their period of initiation in the twenty-ninth hour to their fusion in the fifty-third hour, there occurs a series of cell divisions and movement. These occurrences are made somewhat dramatic when the eyes take on a rose-

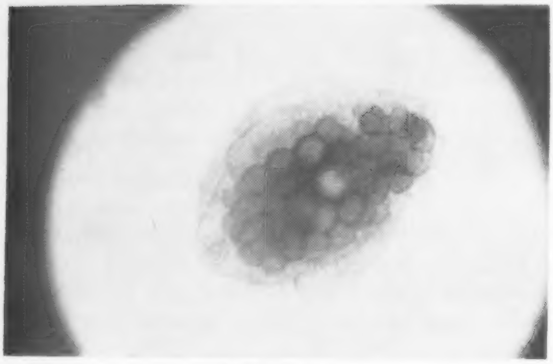

Figure 3

colored pigmentation during the thirtieth hour of development.

A teacher can attract attention and create an interest in this demonstration by pointing out the phylogenetic significance of the eye development. The adult *Simocephalus* has a

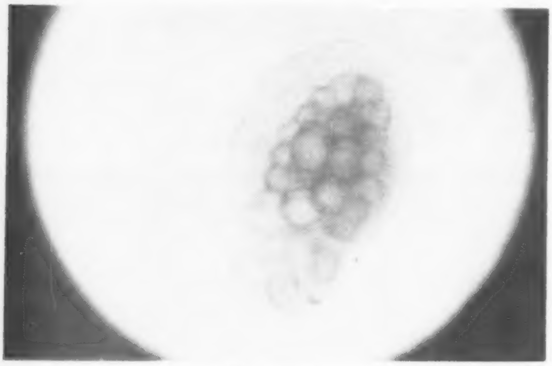

Figure 4

cyclopian eye. However, its ontogeny is of a two-eye nature. It may seem fortuitous to cite the Haeckel concept of ontogeny recapitulating phylogeny.

Figure 5

Figure 5 shows a thirty-seven hour embryo. This photograph shows some cells which have recently undergone cell division. The caudal region exhibits the development of a median line for subsequent bilateral symmetry. The teacher could use this stage as an example of this principle.

The following timetable is included as an aid for the establishment of the stages of *Simocephalus*. Much of the timetable is from an article by Takeo Hoshi, *Science Reports of the Tohuku University*, 4th series, Vol. 19, No. 1, 1951, p. 125.

Stage of Development	*Hours after Deposition of Egg*	*Sequence of Events*
	0	deposition of egg
Gastrula	9	beginning of invagination
Nauplius	18	bursting of egg membrane
	26	appearance of brain cell
	29	appearance of eye cells
	30	appearance of eye pigmentation
	35	pulsation of heart
	36	initiation of body movement
Hatched Embryo	38	moulting of nauplius membrane
Released Young	50	release of larva (free-swimming)
	53	fusion of eyes
	70	first ecdysis

Facts About Strokes

Strokes are not hopeless, and simple rehabilitation measures taken promptly can help many patients return to a useful life. That is the reassuring message of a new leaflet issued by the American Heart Association and its affiliates for the information of the general public. It is available from state and local Heart Associations.

Titled, "Facts About Strokes," the leaflet notes that stroke patients have a more hopeful outlook today because of what can be done by medical science, by the family, and by the patient himself. The most dynamic step in current treatment of strokes "is development of a sound rehabilitation program that will help the patient live and work again to his utmost capacity," the leaflet states.

Use of Amphibians in Advanced Embryology Classes*

ARTIS P. GRAVES

Agricultural and Technical College of North Carolina, Greensboro

Breeding of Some Favorable Species

Natural breeding by various species of amphibia occurs during all seasons of the year. The eggs of many forms, because they are relatively common, may be used for laboratory procedures that do not involve critical operative investigations. Where one is desirous of experimental studies dealing with surgery of the egg or the embryo, the selection of an animal that is quite resistant in these stages of development is essential.

A. *Rana pipiens*

The common leopard frog is most frequently used in laboratories in the United States. However, its short breeding season, March to May, gives one a relatively short span for obtaining eggs, and it is not favorable for studies that extend beyond metamorphosis. O. M. Wolf (7) first reported experimentally induced ovulation in frogs by injection of anterior lobes of the hypophysis. R. Rugh (5, 6) has worked out the standard technique for the method of induced ovulation.

Induced ovulation in our study is accomplished by the injection of fresh or powered anterior lobes of the pituitary gland into the femoral lymph sac and extending the needle into the dorsal lymph sac before releasing the glands from the barrel of the syringe. A size 18 hypodermic needle is used for injections.

B. *Xenopus laevis*

The South African clawed toad spawns the entire year in our laboratory and induced ovulation by the standard technique is possible on any occasion. Since *Xenopus* is entirely aquatic, it is relatively easy to control environmental factors at all stages and extend experiments beyond metamorphosis.

Methods of breeding *Xenopus* have been presented by Aronson (1) and Cameron (2), and more recent breeding methods are described by Chang and Witschi (3).

In inducing ovulation in *Xenopus*, the female is injected with two anterior lobes of the pituitary gland. A male is then injected with a single gland of the pituitary and isolated with the female to await amplexus. Ofttimes males may become stimulated and assume amplexus by the ovulation of the female.

An elevated perforated cover should line the bottom of the aquarium containing the breeding pair as females relish their eggs, and may devour them if this precaution is not followed.

C. *Axolotls*

Axolotls were bred over a three month period extending from January 10, and as late as May 10. During this period, one black axolotl female was successfully mated on four occasions, while a white axolotl had three ovulations. See Table I. In each mating, the same male was isolated with the female, thus indicating a fecundity that makes axolotls a highly desirable form.

Methods

The manner of mating consists of placing together a single well-fed male with a well-fed female in the early evening and using precautions to see that they are left undisturbed. The aquarium is covered with a dark cloth

Figure 1. Courtship of *Pleurodeles*, showing the male ventral of the female with his front leg clamped behind the front legs of the female.

*This work was done while a National Science Foundation Science Faculty Fellow at the State University of Iowa.

and removed the following morning. Gravel in the bottom of the aquarium to serve for attachment of spermatophores increases chances for a successful mating; otherwise spermatophores may be swept aside into corners and make it impossible for the female to become inseminated. Females are removed to smaller, clean aquaria the morning following isolation with males for spontaneous ovulation which occurs within a few hours.

Table I

Breeding Record of *Ambystoma Mexicanum* (The Axolotl)

Mother	*Father*	*Ovulation Date*
M 1 (black)	P 1 (black)	January 10
M 1 (black)	P 1 (black)	March 26
M 1 (black)	P 1 (black)	April 13
M 1 (black)	P 1 (black)	April 23
M 2 (white)	P 2 (white)	March 30
M 2 (white)	P 2 (white)	April 10
M 2 (white)	P 2 (white)	May 10

D. *Pleurodeles*

Our laboratory breeding with the Spanish newt, *Pleurodeles waltli*, extended from January 15 through March 31. During this period two females ovulated twice after a single mating, (see Table II), and in each instance yielded a total of 150-200 fertile eggs. Female 1 had four ovulations, three of which occurred the morning after mating, the other ovulated 9 days after the last mating. Female 2 mated only once during the breeding season, although placed with a mature male on several occasions. Female 3 had three spawnings; two occurring on the morning succeeding the night of mating, the other taking place 18 days after mating. In each situation, females were isolated from males after mating. It is inferred from these mating experiences that the spermatophores remain viable for periods of 9 to 18 days in the cloaca of *Pleurodeles*. Ovulation by cold shock treatment as described by Noble (4), was not effective in our breeding experiments.

Methods

The manner of breeding consisted of isolating a well-fed male and a well-fed female in a large, thoroughly cleaned aquarium covered by a mixture of small and medium size gravel. Repeated spontaneous ovulations depend on the cleaning of the aquarium and its contents at intervals after contamination. Animals are always left in the container during the evening and subsequent night. Females are removed to smaller aquaria the following morning if amplexus has been observed. On several occasions eggs were found attached to the gravel lining the bottom of the aquarium on entering the laboratory the following morning. The ventral position of the male *Pleurodeles* in amplexus has been described by Noble (4), and is shown in Fig. 1.

Table II

Breeding Record of *Pleurodeles waltli* Michah

Mother	*Father*	*Ovulation Date*
M 1	P 1	January 15
M 1	P 1	February 3
M 1	P 1 (x)	February 12
M 1	P 1	March 26
M 2	P 1	February 22
M 3	P 2	February 7
M 3	P 2 (x)	February 25
M 3	P 2	March 31

(x) ovulations occurred without remating with males

Summary

1. A description of the technique in natural breeding of axolotl is presented.

2. A description of successful breeding technique for *Pleurodeles* is given.

3. Breeding technique with *Xenopus* is indicated.

4. The fecundity of axolotls makes them desirable for embryological studies.

5. The prolific tendencies of female *Pleurodeles* is indicated.

6. Retained sperm remain fertile and inseminate eggs of successive ovulations. They have been found viable over periods of 9 and 18 days in *Pleurodeles*.

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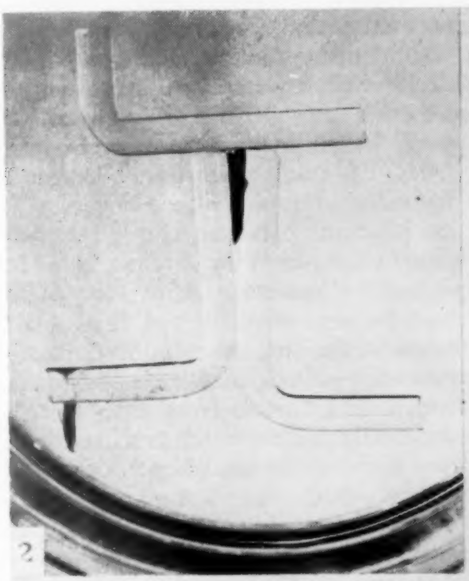
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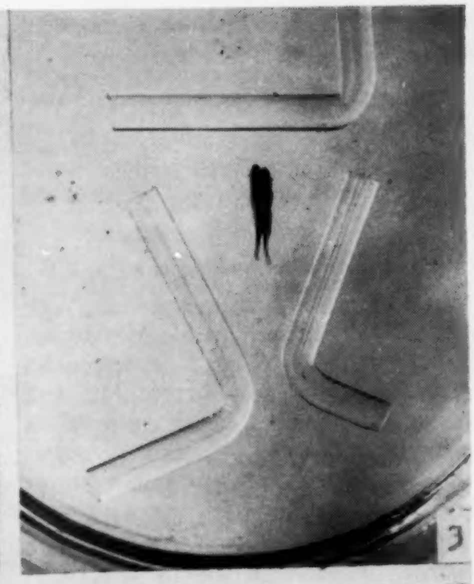
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Experiments on Parabiosis

Many areas of research are possible through the study of amphibian parabiotic twins. Studies that relate to sex development, inductor system, chromatophorotrophic, and other hormonal influences. Since amphibians like the axolotl, *Pleurodeles,* and *Xenopus* are totally aquatic they present one with an opportunity for studies that may extend beyond metamorphosis.

The successful use of a species for parabiosis depends to a certain degree upon the ability of the organism to withstand the injury of the operative processes. It is thought that our experiences with *Xenopus,* axolotl, and *Pleurodeles* might be of some value for those who anticipate their use in embryology classes

Our studies lead us to the selection of *Pleurodeles* as a form most desirable for problems dealing with parabionts.

Materials and Methods

This report is based on the twinnings of *Xenopus, Ambystoma mexicanum* (black axolotl x black axolotl, black axolotl x white axolotl, and white axolotl x white axolotl), *Pleurodeles waltli* x black axolotl and *Pleurodeles waltli* x *Pleurodeles waltli.*

The method of joining was that designed by Witschi (3) and consists of holding the parabionts firmly together between small glass rods (Figs. 2 and 3). Only moderate precautions relative to sterile conditions are necessary. Animals are operated in syracuse watch dishes in a medium of charcoal-dechlorinated water. The epidermis and some adhering mesoderm are removed from the dorso-lateral trunk regions of the mirror surfaces of two embryos at stage 25; the wounded areas are placed together and held in position between the glass rods until permanent fusion by healing is effected.

In some operations with *Pleurodeles,* parabiotic chains were formed (Fig. 4). In these tandem fusions, the injured regions are terminal rather than lateral. After healing, which is effected from one to 12 hours, depending for the most part on the temperature and the mastery of operational technique, embryos are kept in running dechlorinated water until they begin to swim. For a marked degree of success in twinning it is essential that one acquire speed in the removal of tissue from the wounded areas, and in the placement of embryos between the glass rods for fusion.

Eggs are obtained from spontaneous or induced breeding *Xenopus,* or by natural breeding as in the cases of axolotl and *Pleurodeles.*

Discussion

A. *Xenopus*

The relatively small egg of *Xenopus,* the flat embryo of stage 17 coupled with an apparent weak viability in its early developmental stages makes its use quite difficult for typical parabiotic procedures. Chang (1) observed that in side by side parabionts, the tails of twins interfere with the other's motions, causing the animals to sink to the bottom of the container and die in sediment of feces and nettle. To remedy this condition Chang modified the usual operation by removing the front region at the level of the presumptive pronephros of one embryo and

Figure 2. Black axolotl x black axolotl in position for twinning in the glass-rod technique; embryos at stage 30; x 2.

Figure 3. *P. waltli* x *P. waltli* parabionts immediately after the removal of the glass rods; embryos at stage 36; x 2.

Figure 4. *P. waltli* in a triple tandem chain; embryos are joined at stage 26; x 2.

Figure 5. Chang's modified operative technique in pairing Xenopus; embryos are joined when about two days old; x 10.

Figure 6. *P. waltli* x *P. waltli* twins; age 121 days; embryos are joined at stage 26; x 2.

Figure 7. *P. waltli* x black axolotl; 143 days after the parabionts were twinned; embryos are grafted at stage 26; showing the contrast in the gross development of larger *Pleurodeles* and its smaller axolotl twin; x 2.

grafted it to the lateral or the ventral surface of its mate (Fig. 5 and 7).

Since the post-operative mortality in the embryonic stages is quite high in *Xenopus*, a more desirable form for subsequent study was sought.

B. Axolotl

The egg of *Ambystoma mexicanum* is relatively large but soft and not ideally fitted for operative procedures. The embryos are weak at the early tailbud stages, and parabionts at this stage survived only a few days. At stage 30 embryos are more resistant, and by excising the gillbud in addition to the usual dorsolateral trunk epidermis, numerous side by side twins were accomplished at this period, yet most of these die after two or three weeks.

C. Pleurodeles

The eggs of *Pleurodeles waltli* are excellent for operative work in that they develop rather slowly and are quite resistant. Homoplastic side by side parabionts with these forms are successful in about 80% of 100 operations performed (Fig. 6). Xenoplastic parabionts of *Pleurodeles* x black axolotl have survived. In such fusions, the *Pleurodeles* twin seems to outdistance the axolotl in gross development (Fig. 7). In our experiments with axolotl and *Pleurodeles*, the xenoplastic parabionts have a higher survival rate than the homoplastic axolotl combinations. Making allowances for inadequate technique and various factors which might influence the life of the parabionts, it appears that a type of incompatibility may exist in the axolotl x axolotl parabionts.

Summary

1. A description of parabiosis by joining twins through the use of glass rods is presented

2. The weak constitution of the axolotl egg and a possible incompatibility of axolotl parabionts is suggested as a cause of excessive post-operative mortality.

3. The use of *Pleurodeles* for parabiont studies is recommended because of its ability to withstand operative procedures.

4 Xenoplastic grafts of *Pleurodeles* x axolotl have a higher survival rate than homoplastic parabionts of axolotl.

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This investigation was aided by a grant from the National Science Foundation. The author wishes to express his profound gratitude to Dr. Emil Witschi for his invitation and aid in this study which was carried out in his amphibian laboratory. Appreciation is also expressed to Mr. Kazuyu Mikamo, State University of Iowa, for his assistance in the course of this work and for the use of certain material.

Gas Chromatography

Versatile, accurate, and relatively routine gas chromatography techniques which are widely used for analysis of naturally occurring organic compounds have now been successfully adapted in Du Pont Company laboratories to measure minute residues of synthetic biochemicals used in agriculture and animal medicine.

Discussing "Trace Residue Analysis," Dr. Kirkland described the application of general techniques of gas chromatography to determine fractional parts per million of synthetic biochemicals in natural biological samples. Applicable to almost all chemical species, Dr. Kirkland said, the techniques have proved highly selective for a wide variety of specific compounds with a minimum of preliminary isolation and separation steps. Du Pont studies have shown the techniques to be suited uniquely to simultaneous determination of several components, such as isomers, homologs, and "break-down" products during a single operation.

A Unique Growth Pattern for Laboratory Observation

KENNETH SHERMAN,* *Randolph High School, Randolph, Massachusetts*

Introduction

Growth in the biological sciences is a term used universally to denote an increase in the size of a living organism by the assimilation of new matter. Growth most certainly is a basic characteristic of living things.

It is important for the physician to understand the factors affecting the growth of his patients. The forester is concerned with achieving the maximum growth of timber lands in the minimum time. The agricultural scientist is continually experimenting with food crops and livestock in an effort to stimulate growth and increase the agricultural yield. The surgeon standing over a draped lifeless form wonders how long it will be before a vast dedicated group of research scientists develop a method to control and inhibit growth of cancerous body cells. Can the growth of harmful insects be stimulated so that the adult will appear during unfavorable environmental conditions? Entomologists are very much interested in this aspect of growth. The biological oceanographer hopes to someday increase the already rapid growth of marine organisms, while the fishery biologist is concerned with determining the optimum size of fish to ensure a maximum sustained yield for the fishing industries. Yes, growth, or the assimilation of new material by a living organism, is indeed vital to the general health and welfare of the entire world.

Patterns of Growth

While the subject matter concerning growth is wide and diverse, it is possible to demonstrate the basic principle of growth, namely, that a living organism will increase in size by the assimilation of new matter. This can be accomplished by laboratory observation of various growth patterns.

Patterns of growth differ widely among organisms. Man has no particular seasonal increase in size. However, many plants in the temperate regions of the world display a familiar concentric growth pattern which can be seen in a cross section of almost every woody stem in a temperate forest.

Animals that tend to keep their bodies at a constant temperature (mammals) do not generally show a marked increase in seasonal growth. Animals that have difficulty in controlling their body temperature (reptiles, fishes, clams, and a host of others) have a strong tendency toward developing rapidly during periods of favorable temperature, while developing little or not at all during unfavorable conditions of temperature.

The determination of an organism's growth pattern while primarily related to temperature in the temperate regions of the world is also dependent on a number of other factors including the source of energy and the efficiency of its conversion into new matter and also an understanding of the organism's basic ecology, morphology, and physiology.

Observation of Growth Patterns: The Temperate Woody Stem

Growth patterns can be observed in a variety of woody plants. Most all of the trees in a north temperate hardwood forest undergo marked fluctuations in growth. The spring and summer season represent the period of most rapid assimilation of new matter while the fall and winter bring on periods of little or no growth. The leaves are gone, and the radiant energy of the sun is at its lowest level. This seasonal change is recorded by the tree in the formation of concentric rings of large thin walled cells resulting from the rapid spring growth period, while smaller densely packed cells indicate summer growth. The autumn-winter period of sluggishness and absence of growth follows. This type of cellular organization represents an annulus or yearly increase in diameter. The examination of a cross section cut from any hardwood taken from the woodlands of the temperate zone should reveal this pattern.

The Temperate Bony Fish

The determination of a growth pattern is essential to the scientists studying the large

*The author is presently with the U. S. Bureau of Commercial Fisheries, Honolulu, Hawaii.

scale fluctuations in abundance of commercially important fishes.

The fishery biologist utilizes the principle originated by the forester. He is able to determine growth patterns by direct observation of certain bony parts of fishes. The bone most commonly used for this purpose is the sagitta or otolith. This bone is suspended in a watery solution which is encased in a small sinus located in the skull of most fishes. It can be obtained easily by removing the skin and muscle from the dorsal portion of the skull (Fig. 1) beginning at a point parallel to the distal end of the opercle (A) and continuing to the anterior portion of the snout (B). It should not be necessary to remove any tissue lying below a hypothetical transect beginning at the snout, bisecting the eye, and ending at the distal margin of the opercle (B-C).

The removal of the tissue covering the skull will reveal the preoccipital and basioccipital bones which will enclose the otolith. This sinus is located lateral and slightly ventral to the medulla of the brain (X).

The otolith is somewhat oval in shape and more or less pointed at the ends. It functions as a balance organ and aids in orientation of the fish to depth.

After removal, the otolith should be broken midway along its short axis and placed into small clay forms which will provide support. The broken end should be exposed and the pointed end embedded in the clay. They can then be examined under a standard binocular dissecting microscope. Under low magnification alternating opaque and transluscent bands should become apparent. The opaque area represents rapid growth and an increased level of calcium carbonate deposition. The translucent band indicates a period of low level deposition of calcium carbonate usually associated with the fall and winter seasons in the temperate zones of the world.

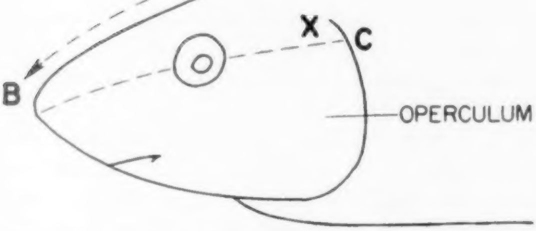

Figure 1. Usual position of the otolith (x) in most bony fishes.

Conclusion

The concept of growth can be illustrated in part by careful examination of growth patterns as they occur in various organisms. Scientific interpretations of these patterns have made it possible for man to increase his yield of living land and ocean resources. Problems, however, continue to exist. Why do cancerous cells take from us vigorous colleagues? Why are organisms endowed with a great variation in size? Can man control growth of living things?

A complete understanding of how and why organisms increase in size by the assimilation of new matter still remains an awe-inspiring unanswered challenge to scientists of the world today. Here is a real challenge for the scientists of tomorrow.

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Depression Drugs

Chemicals derived from the rocket fuel hydrazine have shown powerful stimulating action against depression and fatigue in laboratory animals. The most promising member of the group, 1-phenyl-2-propyl hydrazine, is undergoing extensive clinical trials for the treatment of the depressed state in human beings, Dr. John H. Biel, chief of the chemistry division of Lakeside Laboratories, Inc., Milwaukee, said. The new agents act by directly stimulating the central nervous system and by blocking the brain enzyme that destroys adrenalin and other activating hormones, the chemist explained. The chemical JB-516 has been shown in laboratory tests to inhibit the brain enzyme for an entire week, achieving a remarkably prolonged central stimulatory action, Dr. Biel said.

One of the principal shortcomings of drugs now used to treat depression is that they are too rapidly destroyed by the body's enzyme systems, the speaker pointed out. The presently used drugs are called "sympathomimetic amines" because they mimic the action of adrenalin on the sympathetic nerves. Not only do these drugs have a short period of usefulness, but they tend to lose their effects on repeated administration, to have undesirable depressing after effects, to decrease appetite, and to increase blood pressure and pulse rate, he indicated.

The new hydrazine derivatives produce a prolonged effect with no following period of depression, tend to lower blood pressure and increase appetite rather than decrease it. The success of the tranquilizing drugs brought about a decided change in the philosophy of the treatment of mental disease, the speaker said. It was realized that, by chemically manipulating some of the natural agents in the body, it might be possible to control certain processes that are not functioning properly because of a breakdown in the body's automatic control system.

The Use of Bottled Blood in the Classroom

ROBERT B. SUND
Colorado State College, Greeley

The study of the human circulatory system can be introduced in an interesting manner by an instructor bringing a transfusion bottle filled with whole blood into the classroom. The bottle can be obtained, usually without charge, from the local blood bank and will contain blood which has been stored too long to be used for a transfusion. The blood, however, may still serve many useful instructional purposes and, if refrigerated, kept for more than three weeks.

When displaying a pint of blood, it is desirable to have another transfusion bottle present containing only the nutrient solution. This solution is included in the bottle in order to nourish blood cells while they are being stored. Students by observing these containers filled with the nutrient come to realize that blood is alive and needs food plus all the other substances necessary for life. Some students will undoubtedly notice that the blood does not clot in the bottle. The instructor can use this discovery to develop an understanding of the clotting mechanism of blood and can explain the necessity for using anti-coagulants in transfusions.

A practical application of the principles of air pressure can be illustrated by withdrawing blood from a transfusion bottle. When the instructor inserts a needle into the top of the rubber stopper marked "outlet" on the bottle, no blood will flow from it. This happens because these bottles are partially evacuated before use, and, as a consequence, blood will not flow from them until another needle is inserted into the part of the rubber stopper marked "air inlet" and the bottle inverted. By inserting another needle, air is passed through it and up a glass tube to the top of the inverted bottle. The incoming air causes an increase in air pressure in the bottle which will then force blood to move out the "outlet" opening through a needle and into a syringe or collecting tube.

Whenever blood is taken from the transfusion bottle, sterile techniques should be used so as to preserve it longer. Furthermore, the demonstration of the aseptic method in withdrawing blood from the transfusion bottle may be used to stimulate classroom discussion about aseptic and antiseptic techniques.

The demonstration of oxygenation of blood can be shown by withdrawing part of the blood from the transfusion bottle with a needle and syringe or by using the plastic tube and needles which come with a regular transfusion bottle. Place the collected blood in a large test tube. Pass oxygen through it either from a small gas cylinder or from some chemical source. The blood will turn a fairly bright red. Fill another tube in the same manner and insert a hollow glass tube in it through which carbon dioxide gas is passed either from a cylinder or by a student blowing his breath through the tube. The blood will change color to give a typical venous appearance. These two tubes can be compared to show the appearance of the red oxyhemoglobin and the bluish desoxyhemoglobin. After observing the demonstration, students should be asked to look at the color of their veins and compare them with the color of the blood in the test tubes. By making such a comparison, they can conclude which of the two types of hemoglobin their veins carry.

Some of the other activities that may involve the utilization of bottled whole blood are:

1. Use it to determine the pH of blood and to study its buffer action.
2. Take out some of the blood periodically and study the cells under the microscope in order to determine if any of them change in appearance with age.
3. Count periodically, by use of a counting chamber, the blood cells and calculate their life expectancy.
4. Make a blood agar culture by placing 5-10 cc. of sterile blood on an agar plate. Inoculate the plate in order to determine whether bacteria will grow on it.
5. Take some of the blood from the bottle and blood type it. Demonstrate how blood typing is done and relate this to questions about biological differences and the inheritance of blood types.

6. Refer to the small tube which is normally attached to the transfusion bottle for serology testing. Its presence may be used to illustrate the fact that blood can carry infectious organisms.

7. Place a transfusion bottle in an undisturbed place for a number of hours so that the cellular portion of the blood settles. By observing the bottle students can determine the relative proportion of plasma in blood.

Instructors who use whole blood from transfusion bottles as a teaching material should emphasize that such a use is not wasteful. It should be pointed out that blood is almost always utilized for its intended purpose before it expires. It should be further mentioned that even expired blood can be used to make plasma or as culture media in some bacteriological tests.

Bathtub Ecology for the Biology Class

CHARLES D. WISE,* *Institute of Marine Science, The University of Texas, Port Aransas*

At the Institute of Marine Science old bathtubs have been found useful in making artificial temporary ponds for studying various ecological aspects of freshwater environments. Old bathtubs can often be bought from junk yards at very reasonable costs and at considerably more attractive prices than regular glass aquaria. Techniques employing bathtub ponds can have wide applications. For example, in areas where a large part of the year is dry and the area devoid of any readily available freshwater ponds, such techniques could be highly successful in pointing up certain fundamentals of biology. In winter, indoor bathtub ponds can be adjusted by proper addition of light, nutrients, etc., to simulate conditions of spring and bring about the burst of life characteristic of this season. This can make a profound and lasting impression on students in the biology classroom. In addition to the effect of light, temperature, and nutrients on the environment, plants and animal succession and variety may be demonstrated. For example, in one bathtub culture set up at the Institute of Marine Science, the following was observed:

After two days—numerous cladocerans and copepods.
After three days—numerous conchostracans and a heavy *Volvox* bloom.
After four days—numerous ostracods.
After twelve days—gravid female ostracods.
After fifteen days—fairy shrimp and tadpole shrimp, *Volvox* disappearing; ostracods mating.
After sixteen days—appearance of mosquito larvae.
After seventeen days—colonial rotifers.
After one month—*Chara*; a heavy mat of *Pithophora* forming.
After two months—*Pithophora* starting to drop out; a heavy growth of *Chara*.

The technique is simple. Merely place an inch or two of soil, dried mud, or sand from an evaporated pond or ditch area in the tub and add several inches of distilled or rainwater. Tap water may be used if first freed of chlorine, although results may not be as good as with rain water or distilled water. After a few days, life in various forms will start making its appearance, and the resistance of spores, eggs, and cysts to desiccation will be demonstrated in a most positive manner. A plastic cover for the tub will serve to prevent excess loss of water due to evaporation. The water may be kept at a certain level by the addition of more as needed, or the tub may be allowed to lose water steadily until desiccated. A succession will continue through this period, and the whole order can be started again by the simple addition of water.

If a small electric pump is available, streams can be simulated by running water over plank or rock inclines installed in the tub. Such "streams" often develop their own characteristic, and highly interesting, algal floras.

Many of the experiments in basic ecology as outlined by H. T. Odum in the February, 1960, issue of *The ABT* can be performed by the biology class using such an artificial pond. This may be especially important in

*Present address: The University of New Mexico.

areas where a natural pond is not readily accessible and the time element is critical. E. P. Odum's textbook is recommended for additional reference on the fundamentals of ecology, many principles of which can be demonstrated by bathtub ecology. References on aquaria and the identification of freshwater organisms may be found in the article on oceanography by the present author which appeared in the December, 1959, issue of *The ABT*.

The technique lends itself to many uses and varying approaches. The artificial pond can be used as a source of ready biological material for study in the classroom. By varying conditions of light, temperature, and nutrients, the importance of these factors in the environment can be demonstrated. Productivity of ponds can be shown through determination of oxygen and carbon dioxide production by the community by day and night. Resistance of spores, eggs, and cysts can be demonstrated positively by the simple addition of water to dried soil or mud. Succession which occurs naturally through the seasons outside can be seen right in the classroom. In brief, bathtub ecology is much fun, inexpensive, and offers unlimited opportunities to show basic biological and ecological principles.

References Cited

Odum, E. P. with the collaboration of H. T. Odum. 1959. *Fundamentals of Ecology*. Saunders, Philadelphia.

Odum, H. T. 1960. Ten classroom sessions in ecology. *The American Biology Teacher*, 22(2): 71-78.

Wise, C. D. 1959. Dear Student: Are you interested in oceanography? *The American Biology Teacher*, 21(8): 341-346.

Book Reviews

GENERAL BIOLOGY, LABORATORY GUIDE, A. Paul Davisson, 165 pp., $3.50, Burgess Publishing Co., Minneapolis, Minnesota, 1960.

This is a fairly orthodox manual for elementary college biology courses. The reviewer assumes it is for a one-semester course. The frog is the main type animal used. The author believes that furnished drawings are superior to those of the student. There are some simple experiments also included. Both plant and animal kingdoms are taken up in phylogenetic sequence after the study of the frog.

P.K.

THE BIOLOGY OF WEEDS, John L. Harper, 256 pp., $9.75, U. S. representative: Charles C. Thomas Co., Springfield, Illinois. Blackwell Scientific Publications, Oxford, England, 1960.

Perhaps most teachers would agree that field work is a desirable part of a biology course, but for one reason or another they may fail to get their students out of the classroom, the reason quite often being that suitable areas for field trips may be some distance from the school. The desirable area for field trips are often thought of as a woods, a bog, or some other type of natural habitat where wild flowers grow in profusion. These teachers may be overlooking a "weed patch," conveniently located in a nearby vacant lot, along a roadside, or in an abandoned garden. This is to be regretted because weeds provide excellent teaching materials and may serve to illustrate some biological principles better than the "natural" plants. Several works are available dealing with the identification of weeds and weed control, but few books have touched upon other basic aspects of weeds, so the appearance of "The Biology of Weeds" is indeed welcome. This work is the result of a symposium of the British Ecological Society held in 1959, and the twenty-five papers devoted to weeds cover such topics as taxonomic problems, dormancy and dispersal of weeds, population studies, including interference and competition, autoecological studies, and special problems in connection with certain groups of plants. The authors for the most part are English, and their discussions are confined largely to European weeds. One will find, however, that many of these same plants are among the most common and familiar weeds in the United States. One brief criticism is in order. Although most or the articles have adequate bibliographies, some important American contributions bearing on the subjects discussed have been overlooked. Some of the articles will be of interest primarily to agricultural botanists, but most of them are of more general interest. It is obvious from the

book that the interactions between man, weeds, and natural vegetation is a most complex subject and that while a good start has been made on answering many questions, much is yet to be learned.

Charles B. Heiser, Jr.
Department of Botany
Indiana University

WORKBOOK FOR WOODY PLANTS, James W. Hardin, 131 pp., $3.50, Burgess Publishing Company, Minneapolis, Minnesota, 1960.

This is a laboratory manual for a course in dendrology, but it will have real usefulness to the high school biology teacher. There is a complete listing and description of all the terminology used in woody plant taxonomy. The bulk of the book is devoted to a descriptive listing of the genera which include woody plants from the monocots to the gymnosperms. Species are listed but without descriptions under each genus. Useful in taxonomic work above its original purpose as a dendrology lab manual.

P. K.

THE PLANT KINGDOM, Harold C. Bold, 114 pp., $1.50 (paperback), Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 1960.

This book, another in the Prentice-Hall Foundations of Biology series, is a survey of the plant kingdom with the primary emphasis on morphology. Numerous line drawings and photographs are included. The treatment is essentially that adopted in a number of college botany textbooks. The book provides an excellent over-all view of the plant kingdom and would be a good source of supplementary reading for the high school biology student. It should be pointed out that the author also has a textbook, *Morphology of Plants*, (1957), which covers the same subjects in considerably greater detail.

THE LOWER ANIMALS, Ralph Buchsbaum and Lorus J. Milne, 303 pp., $12.50, Doubleday and Company, Garden City, New York, 1960.

An absolutely stirring book and meritorious addition to the *World of Nature* series of this publisher. No person can look through the gorgeous illustrations in full color without becoming entranced with the world of invertebrates.

The authors are famous in the field of invertebrate zoology, but this reputation is enhanced by the fact that many of the beautiful photographs are their work, especially that of Professor Buchsbaum. Their writing style is easy, informative, and replete with entertaining facts about each animal. There are no diagrams with the familiar labels found in most texts.

Animals are described in detail which seldom even appear in elementry zoology texts. The book has chapter headings which have phylum names as subtitles and common terminology for main heads. These chapters number twenty, and this will give one an idea of the scope of the book. One chapter even includes several phyla. The chapters are then subdivided into the main orders. Separate genera and species are described in detail. But most interesting are the details of the lives and habits of these animals and their pictures.

This is the kind of reference book which should be on biology and general library book shelves in schools and colleges. It is not a text, but it is the type of reference students will delight in using, looking through, and simply reading for the sheer fascination of the information included.

P.K.

AIDS TO HISTOLOGY, Seventh Edition, G. H. Bourne, 167 pp., The Williams & Wilkins Co., Baltimore, Maryland, 1960.

This pocket-sized hard-bound book is precisely what the title indicates—aids to histology. The book is clearly and simply written, developing a brief account of the histological structure of the mammalian body. It is recommended as a supplemental text for courses of physiology and histological technique where an adequate knowledge of histology has not been obtained.

L. L. Hearson
Park College
Parkville, Missouri

LYMPHATICS AND LYMPH CIRCULATION, Rusznyak, Foldi, and Szabo, 853 pp., $20.00, Pergamon Press, Inc., New York, 1960.

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phatics and other lymphoid tissue. The authors have seen fit to divide the work into three parts: origin and anatomy, general physiology and pathology, as well as special physiology and pathology of the lymphatic system. All of this material is spread out in the text of 853 pages and 252 well-chosen illustrations, many in color. The translation and publication from the original Hungarian has been a monumental task, and the authors have made nearly as complete a survey of the field as Rouviere, Drinker, Kampmeier, Clarks, and many other investigators. The inclusion of the writers' first-hand investigations of points which have been controversial contributes to the originality of the book.

Obviously, this is not a text for undergraduate students, but it should be helpful to advanced graduate students with a biological background and to investigators in the field of lymphoid and lymphatic tissue.

R. L. Webb
Department of Anatomy and Physiology
Indiana University

BEHAVIOR GENETICS, John L. Fuller and W. Robert Thompson, 396 pp., $8.95, John Wiley & Sons, Inc., New York, 1960.

This is a fine, new treatment of a vast body of literature heretofore uncollected and unevaluated in a comprehensive way. The field of the genetic studies of behavior extends from plants to humans. The style of the volume is to compile many isolated studies by biologists and psychologists.

The book is divided into three parts: an introductory section on elementary genetics, a critical review of the literature of animal and human studies, and a synthesis of the current knowledge with a proposal of a new theoretical framework for future research.

The chapters in the critical review section are in sensory and perceptional processes, response processes, intellectual abilities, personality and temperament, and mental disorders. This listing will serve to show the extensiveness of the literature. But the concluding section makes clear an implication throughout the book that many behaviorial patterns are inherited. The studies used make a mass of data which cannot be ignored. Whereas behavior is often talked about as having *some* type of genetic background, this book makes clear much of the research which suggests